TissueFAXS 3.0

User Manual

Table of Contents

1.1. Purpose 7
1.2. Goals 7
1.3. Definitions, Acronyms and Abbreviations 8
1.3.1 Definitions 8
1.3.2 Acronyms 8
1.3.3 Abbreviations 8
1.4. Conventions 8
2.1. Installation Overview 10
2.2. .NET Framework 2.0 Runtime 10
2.3. MTB RDK 1.6.0.5 10
2.4. Intel Performance Primitives 5.3 11
2.5. Microsoft Visual C++ 2005 Redistributables 11
2.6. TissueFAXS 11
2.7. System Requirements 12
2.7.1 Hardware Requirements 12
2.7.2 Software Requirements 12
3.1. Login Dialog 13
3.2. Splash Screen 14
3.3. Device Calibration 14
4.1. Application Options 16
4.1.1 Actions 16
4.1.2 Licensing 19
4.1.3 Measurements 20
4.1.4 Remember 24
4.1.5 Support 24
4.2. Default Experiment Settings 28
4.2.1 Preview 28
4.2.2 Reflectors 29
4.2.3 Storage 30
4.3. Scan Settings 32
4.3.1 Scan Strategy 32
4.3.2 Stitching 32
4.3.3 Focus 34
4.3.4 Advanced Focus 38
4.3.5 Speed 39
4.4. Hardware Settings 40
4.4.1 Camera Manager 40
4.4.2 Contrast Manager 40
4.4.3 Stage Manager 41
4.4.4 Load Settings 48
4.5. Experiment Options 48
4.5.1 Preview 48
4.6. Calibrate Field of View 49
4.6.1 Manual FOV Calibration 50
4.6.2 Automatic FOV Calibration 54
4.6.3 Distance Calibration 57
4.7. Users 61
4.7.1 View Users 61
4.7.2 Adding Users 62
4.7.3 Modifying Users 63
4.7.4 Delete Users 63
4.7.5 Change Password (Administrator Mode) 63
4.7.6 Role Based Administration 64
5.1. New Experiment Wizard 66
5.1.1 Experiment Creation 67
5.1.2 Browse for template/experiment 69
5.1.3 Experiment Identification 70

5.1.4 Experiment Settings 71
5.1.5 Finish: Experiment Ready 73
5.2. Preview Settings 74
5.2.1 Brightfield Experiment 74
5.2.2 Fluorescence Experiment 75
5.3. Acquisition Settings 76
5.4. Standard acquisition vs Time Lapse acquisition 80
5.5. Microscope 84
5.5.1 Objectives 85
5.5.2 Reflectors 85
5.5.3 TL Shutter for transmitted light 88
5.5.4 TL Lamp 89
5.5.5 RL Shutter for reflected light 89
5.5.6 Fluo Arc 89
5.5.7 White Light Attenuator (optional component) 90
5.5.8 Fluorescence Attenuator (optional component) 90
5.5.9 Condenser 90
5.5.10 Condenser Front Lens 91
5.5.11 Condenser Aperture 91
5.5.12 Sideport Changer 91
5.6. Stage 91
5.7. Cameras 93
5.7.1 Adding Cameras 93
5.7.2 Change Default Camera 95
5.7.3 Operations with Current Camera 95
5.7.4 Remove Camera 95
5.7.5 Settings for PCO/PixelFly Camera 95
5.7.6 Settings for PixeLINK Camera 99
5.8. Camera Panel 103
5.8.1 Stage control 103
5.9. Camera Control 104
5.10. Drag-drop panel 111
5.11. Camera settings for Preview and Acquisition 111
5.12. Making a preview 111
5.13. Stopping a Preview 113
5.14. Experiment Editor 113
5.15. Slide Manager 119
5.16. Slide Editor 121
5.16.1 Add Region 123
5.16.2 Modify Region 125
5.16.3 Copy and Paste region 127
5.16.4 Preview Area 128
5.16.5 Stage Menu 129
5.16.6 Export this slide image… 133
5.16.7 Automatic tissue detection 133
5.16.8 Slide Overlay 136
5.16.9 Custom Selection 136
5.17. Region Viewer 137
5.17.1 Subregions 139
5.17.2 Categories 140
5.17.3 Annotations 143
5.17.4 Illumination Correction (Shading Correction) 145
5.17.5 Flags 149
5.18. Field of View Viewer 157
5.19. Field of View Viewer - Overlay feature (Fluorescence) 158
5.20. Export 160
5.20.1 Export FOVs Images 160
5.20.2 Export Region Overview 162
5.20.3 Export One FOV Image 167
5.20.4 Export current Slide Image 169
5.20.5 Export Annotations 170
5.21. Use Plugins 172

6.1. Automatic detection of TMA structures 173
6.2. Manual definition of TMA structures....175
6.2.1 Definition of TMA block size....175
6.2.2 Definiton of the first TMA block on slide....177
6.2.3 Definition of the second TMA block on slide....178
6.2.4 Selection of TMA blocks and spots....179
6.2.5 Resize the TMA spots....180
6.2.6 Rotate a TMA block....181
6.2.7 Adjust the distance between TMA spots....182
6.2.8 Moving TMA blocks and spots....183
6.2.9 Deleting TMA blocks and spots....183
6.2.10 View acquired images for TMA spots....184
6.2.11 Grouping TMA spots....184
7.1. Print Options....187
7.2. Print Items....188

Disclaimers

1. **TissueFAXSplus** is a microscope-based cell analysis system for cells in cryocuts, paraffin sections and TMAs. It consists of the software modules "**TissueFAXS**", "**TissueQuest**", and "**HistoQuest**" and is used for acquisition of images in the fluorescence and brightfield mode, for counting the number of positive and negative cells and for quantification of staining intensities. **TissueFAXSplus** is used for the standardization of tissue analysis in combination with immunohistochemical and immunofluorescence staining.

The system does not give any direct diagnosis and there is the possibility that the samples do not contain enough information to give a clear diagnosis.

The results of the analysis are purely statistical values. Users must reevaluate images and likelihood of the statistical data. Pure interpretation of statistical data is a high risk.

Observations:

- **TissueFAXS** is similar to **TissueFAXSplus** but is for fluorescence samples only and must not be used with brightfield/immunohistochemical samples. **TissueFAXS** consists of the software modules "**TissueFAXS**" and "**TissueQuest**".

- **HistoFAXS** is similar to **TissueFAXSplus** but is for brightfield/immunohistochemical samples only and must not be used with fluorescence samples. **HistoFAXS** consists of the software modules "**TissueFAXS**" and "**HistoQuest**".

2. Each and any product should be used only after training performed by TissueGnostics or authorized distributors of TissueGnostics. A list of authorized distributors is available here:

http://www.tissuegnostics.com//index.php?load=xml/en/home/company/distributors.xml&lang=en

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4. In order to use **TissueFAXS** application, it is essential for the users to have sufficient knowledge of PC and Microsoft Windows operating system usage.

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10. Release date of this document: ***17 June 2011***

11. Document version: ***3.5***

Notices about symbols and labels on product

The following symbols appear on the labels of the product:

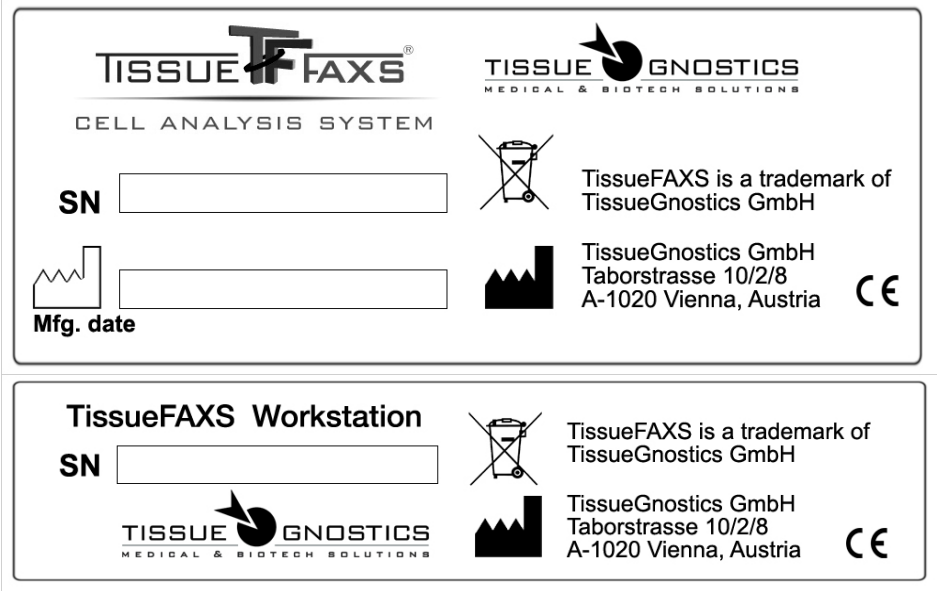


Figure 1 - Product labels

SN Serial number

WEEE symbol / separate disposal of electrical and electronic equipment

Date of Manufacture

CE mark

Manufacturer

Notice regarding anti-virus applications

- All the TissueGnostics products are shipped with the ***Kaspersky Anti-Virus*** in order to ensure optimal functionality.
- Therefore TissueGnostics ***only guarantees for Kaspersky Anti-Virus*** to work with its products.
- The user may decide to use other anti-virus software only upon own responsibility.
- Any problem that arises due to other anti-virus software installed will ***void TissueGnostics warranty*** and every support or service action required by the client in order to restore any of TissueGnostics damaged instruments will be billed to the customer.

1. Introduction

1.1. Purpose

The purpose of this document is to help the user understand how **TissueFAXS** works. It describes **TissueFAXS** software capabilities and also guides the user to the steps required to acquire high quality images.

The document covers the installation of the application, system requirements and main features.

In the next section you will read more about the goals of the application and its place in a larger system. You will also understand the purpose and the advantages of this application.

1.2. Goals

The user should use this manual as his primary source of instruction when using the application. The functionality of all panels, menus and tools are explained in the following tutorials.

At a glance, **TissueFAXS** provides:

- Fully automated slide scanning;
- Autofocus up to 63x Oil objective;
- Multi-channel acquisition for fluorescent imaging;
- High resolution image overview by TissueStitching;
- Freely selectable areas of interest;
- Channel overlay and data storage.

Features of **TissueFAXS**' automated image acquisition:

- Slide readout;
- Pre-scanning to identify tissue sections, smears and biopsies;
- Multi-slide management and stage movement;
- Self adjusting color and monochrome CCDs;
- Automated objective lens and filter block handling;
- High-speed and high-precision autofocus;
- TissueStitching for overviews of entire sections;
- Time Lapse acquisition;
- Channel overlay and data storage.

TissueFAXS rapidly yields high resolution images of tissue sections for subsequent analysis in **HistoQuest** or **TissueQuest** (the cell analysis software for brightfield and fluorescent images, respectively).The process of image acquisition to data analysis and quantification are streamlined with the TissueGnostics software combination. Quick and easy data analysis in **HistoQuest** and **TissueQuest** is accomplished with these key features:

- Powerful algorithms for image processing;
- Automated identification of cells;
- Analysis of staining intensities;
- FACS-style dot plot operations;
- Backward connection from dot to cell;

- Storage of virtual slide data and analyzed results.

TissueFAXS enables the user to link all experimental image files with their corresponding data files into one convenient experiment folder.

TissueFAXS supports upright and inverted microscopes from various manufacturers including Leica, Nikon and Zeiss. This manual was designed for the use with Zeiss microscopes, but it is transferable by a large extent to other supported microscopes.

1.3. Definitions, Acronyms and Abbreviations

This subsection provides the definitions of all terms, acronyms, and abbreviations required to properly interpret this Document.

1.3.1 Definitions

Term	Definition	Comments
	-	-
-	-	-
-	-	-

1.3.2 Acronyms

Term	Definition	Comments
IKF	**Installation Kit Folder.** The **IKF** is a folder where you find a set of executable files. These files are kits for installation of certain components. When you install **TissueFAXS**, you go to this folder and you find a list of executable files, which you must execute one by one. See *Installation Overview* for further information.	-
MTB	**Microscope Tool Box.** The **MTB** from Carl Zeiss, a software component which gives an identical interface for all Zeiss microscopes under different operating systems.	-

1.3.3 Abbreviations

Term	Definition	Comments
-	-	-
-	-	-

1.4. Conventions

In this section you can find listed and explained the conventions used throughout this manual.

Note(s): describes important features or instructions.

Tip(s): highlights features or hints that can help you save time or avoid difficulties.

Caution(s): warns readers about possible damage to equipment or to data or about potential problems in the outcome of what is intended.

Italics: are used in order to highlight a title and also new or unfamiliar terms, usually at their first appearance in the text.

Bold: are used in order to highlight important terms or to mark the names of the features/GUI elements throughout the text of this manual.

→: the arrow indicates a menu choice. For example, choose "**File** → **Save as template**" means you have to choose **Save as template** from the **File** menu.

2. Installing TissueFAXS

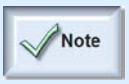
2.1. Installation Overview

Where do I find the installation kits for the required components?

Usually, the **TissueFAXS** installation kit is provided with a set of other installation kits for the rest of the components, which can be found in the same folder. Most of the components are also found on official websites. You need an internet connection in order to download those components.

In order to make the installation procedure easier, you are provided with a folder where you find all required kits. Install them in any order you want, but make sure **TissueFAXS** is the last one to be installed. If you are not sure how to start, try this order:

1. NET Framework 2.0 Runtime with Service Pack 2;
2. Microsoft Visual C++ 2005 Redistributables;
3. Intel Performance Primitives 5.2;
4. MTB RDK 1.6.0.5;
5. **TissueFAXS**.



- **TissueFAXS** software will not run and operate properly if any of the components is not installed on your **TissueFAXS** workstation.
- Installing software programs and *Windows* system components will require *Administrator* privileges on your computer. You might need to ask for support at your IT department if you do not have privileges to install software.

2.2. .NET Framework 2.0 Runtime

The **TissueFAXS** product is entirely built over the version 2.0 of .NET Framework which can be also found at the Microsoft official website.

The Microsoft .NET Framework version 2.0 (x86) redistributable package installs the .NET Framework runtime and associated files required to run applications developed to target the .NET Framework v2.0 (TissueFAXS is such an application).

For best results at least .NET Framework SP2 must be installed.

For further information about this package please visit the *Microsoft* homepage.

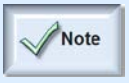
2.3. MTB RDK 1.6.0.5

You find this kit in the *Installation Kit Folder* (IKF) of **TissueFAXS**.

TissueFAXS requires this installation to communicate with the microscope.

Below are some features of the Micro Toolbox (MTB):

- Gives an identical interface for Carl Zeiss microscopes under different operating systems;
- Has high level functions for controlling the components;
- Gives information about the equipment (e.g. which objectives and reflectors are mounted);
- May simulate devices: programming and demos are possible even without hardware.



- For problem solving and re-installation, it is essential that you configure your microscope in terms of “teaching” it which hardware is provided. To do so we

recommend using the MTB configuration tool. Do not forget to write the configuration into the microscope hardware when you are finished. Present hardware that was not enlisted in the configuration will not be accessible!

- The **TissueFAXS** instrument you received from TissueGnostics or your local distributor has been preconfigured and you should not change anything in this configuration, unless you encounter technical problems.

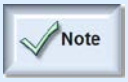
2.4. Intel Performance Primitives 5.3

Intel® Integrated Performance Primitives (Intel® IPP) is a software library for application developers, providing better performances on Intel's latest microprocessors. Incorporating these functions into the **TissueFAXS** software will ensure that the potential of your Intel-based PC can be fully exploited for analysis of your samples.

2.5. Microsoft Visual C++ 2005 Redistributables

The Microsoft Visual C++ 2005 Redistributable Package (x86) installs runtime components of Visual C++ Libraries required to run applications developed with Visual C++ on a computer that does not have Visual C++ 2005 installed.

TissueFAXS contains modules that were developed with Visual C++, so this Redistributables kit is required.



- **IMPORTANT technical note**: If you have installed previous pre-release versions of Visual C++ 2005 or Visual Studio 2005, such as Beta 1, Beta 2 or Community Technical Preview (CTP) builds, then you must uninstall these versions via *Add/Remove Programs* in *Control Panel* before installing the final released version.
- The **TissueFAXS** instrument you received from TissueGnostics or your local distributor has been preconfigured and you should not change anything in this software /hardware configuration, unless you are directed by the support team.

For further information about this package please visit the *Microsoft* homepage.

2.6. TissueFAXS

The **TissueFAXS** installation kit is the last file installed from this list of required components.

It consists of a single executable file. Once launched, it leads to a well known installing procedure.

You just need to run it and follow the instructions.

Mainly, you have to choose the destination directory and that's all.

An important feature is that different versions of **TissueFAXS** may coexist on the same machine.

You only need to make sure that they are installed in different directories.

If you want to install the same version of **TissueFAXS** in the same directory, please uninstall the existing version first, instead of overwriting it. This ensures that no files are in use and the new installation will be a fresh new one.

Also note successive installations of **TissueFAXS** do not require re-installation of the other components as well. Once installed, the other required components work independently of **TissueFAXS** and keep working, unless you have uninstalled each one by one.

TissueFAXS is ready to use when all components are installed and there are no error messages.

Firstly, turn on the hardware: turn on the microscope and the UV-lamp, if required. Secondly, turn on the computer and run **TissueFAXS**.

2.7. System Requirements

2.7.1 Hardware Requirements

Minimum

- Intel Pentium 3 Processor - 1 GHz
- 1024 MB RAM
- USB-Port (for dongle)
- 100 GB available hard disk space
- Single monitor system with a resolution of 1024x768

Recommended

- Intel Core2Duo Quad Processor - 3 GHz (or more)
- 4 GB RAM
- Internet connection (for update)
- USB-Port (for dongle)
- 1000 GB hard disk space for storing your projects
- Dual monitor system with a resolution of 1280x1024 (or higher)

2.7.2 Software Requirements

- Windows 2000
- Windows XP
- Windows Vista x32
- Windows Vista x64 (only for brightfield experiments - not compatible with PCO camera)
- Windows 7x32 (recommended)
- Windows 7x64 (only for brightfield experiments - not compatible with PCO camera)

Notice regarding virtual machines

TissueGnostics software is not meant to work on virtual machines. Also, TissueGnostics does not offer support for these configurations, as the software directly uses advanced CPU features and therefore any third layer between the software and the CPU will affect performance and might affect the results of the analysis in an unpredictable way.

3. Starting TissueFAXS

3.1. Login Dialog

After starting the application, a login panel will appear. In order to login to **TissueFAXS**, you must enter a username into the **User Name** field and type the proper password into the **Password** field.

Press **I Agree and Login** to be authentified. By pressing this button you explicitly agree to the *TissueGnostics Disclaimer*.

If the username and password are correct, the login dialog will close and you can start using the application. Otherwise, the application will prompt again for a valid username and password combination.

If you press **Cancel**, the login dialog and the application will close.

The login dialog is used to restrict access to the application. There is one Administrator and any number of users, who may be managed when the Administrator is logged on (for more details please see **Chapter 4.7**).

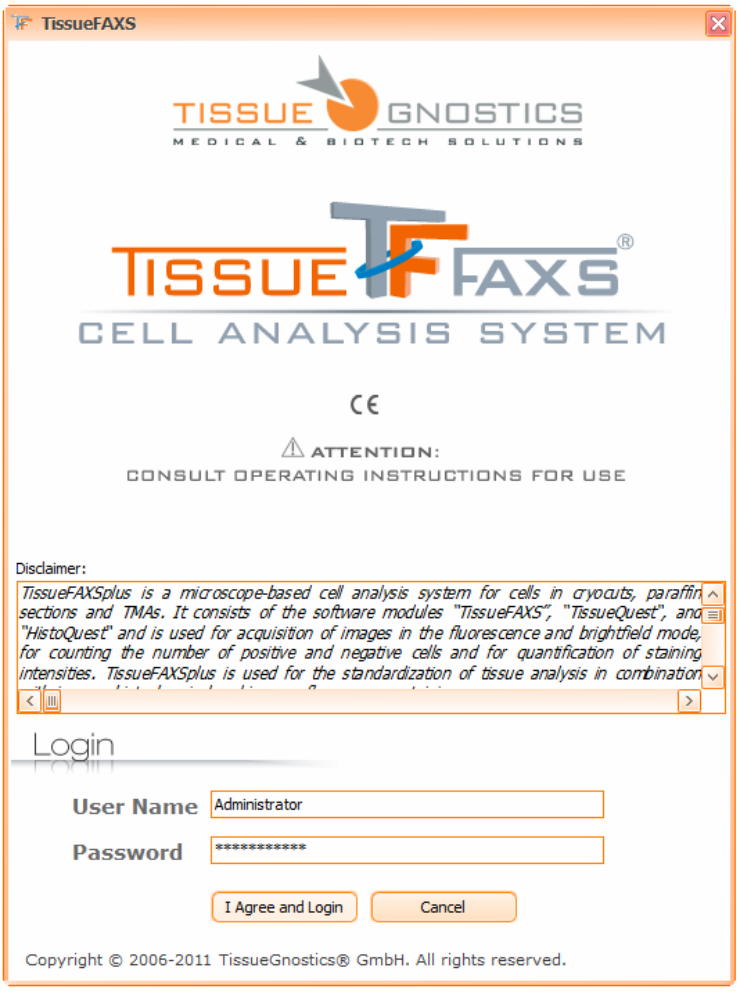


Figure 2 - Login Panel

3.2. Splash Screen

The **Splash Screen** appears while **TissueFAXS** loads and establishes communication with the hardware components (microscope, stage, cameras).

Figure 3 - Splash Screen

3.3. Device Calibration

To start an image acquisition, you must choose to calibrate the stage when starting the application. You may choose to skip this step if you simply want to view previously acquired images in **TissueFAXS**. If you later decide to perform acquisition, you will be prompted to calibrate the stage at that time.

Stage calibration is important for acquiring high quality stitched images, as well as the protection of the hardware components from physical damage. During the calibration, the application finds the hardware limits with extreme precision. You will see the objectives and stage moving automatically, as the system measures the coordinates of the stage in relation to each objective (in nanometers).

To initiate the calibration procedure, press **Continue**.

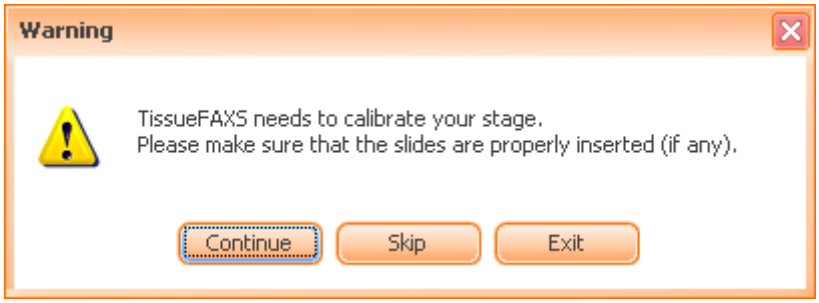


Figure 4 - Stage Calibration warning message

If the magnification of the calibration objective is higher than 20x, a warning message will appear. In this situation there are two options:

- Press **Cancel** calibration, then go to **Tools** → **Options** → **Application Options** → **Actions** → **Calibration** in order to select another objective from the dropdown list, then return to calibration process from **Tools** → **Calibrate stage...** For more details, please see **Chapter 4.1.1.**
- Press **OK** to continue calibration.

- If you continue the calibration as described above, you should think about the risks, such as damages to your objective, stage or slides.

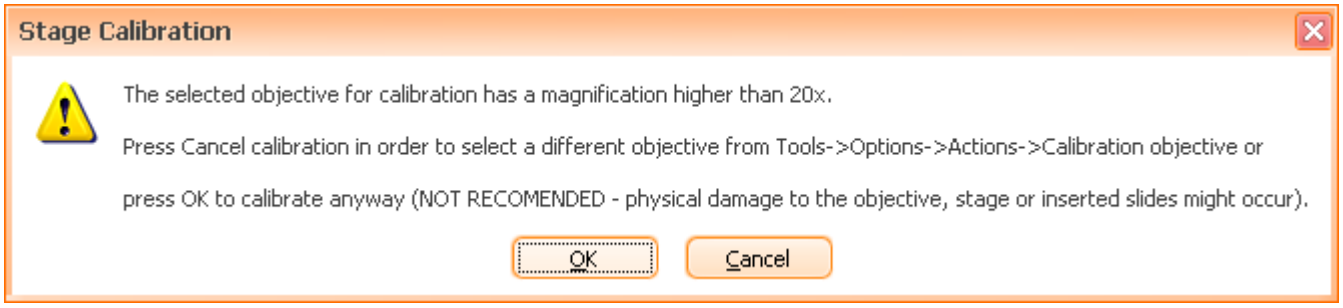


Figure 5 - Calibration objective: warning message

After the calibration ends, **TissueFAXS** is ready to be used. You can now create new projects, open existing ones, or simply adjust the microscope components and see live images from the camera, as described in the following chapters.

4. Options

The **Options** panel is very useful in managing the main utilities of the application. Here, by accessing the listed items, you can visualize the available settings and also modify them according to your needs.

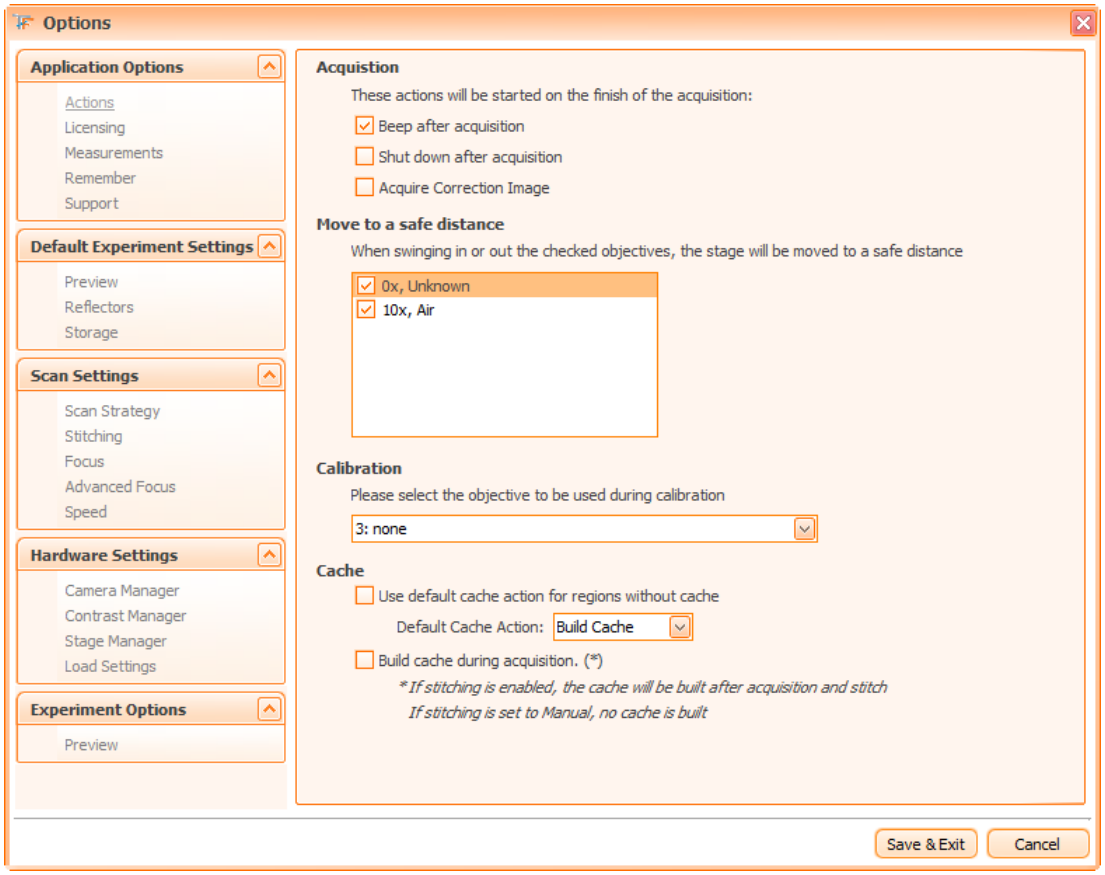


Figure 6 - Options Panel

4.1. Application Options

4.1.1 Actions

This section contains several important actions concerning the interaction between **TissueFAXS** and the other components of your system: the computer and the microscope.

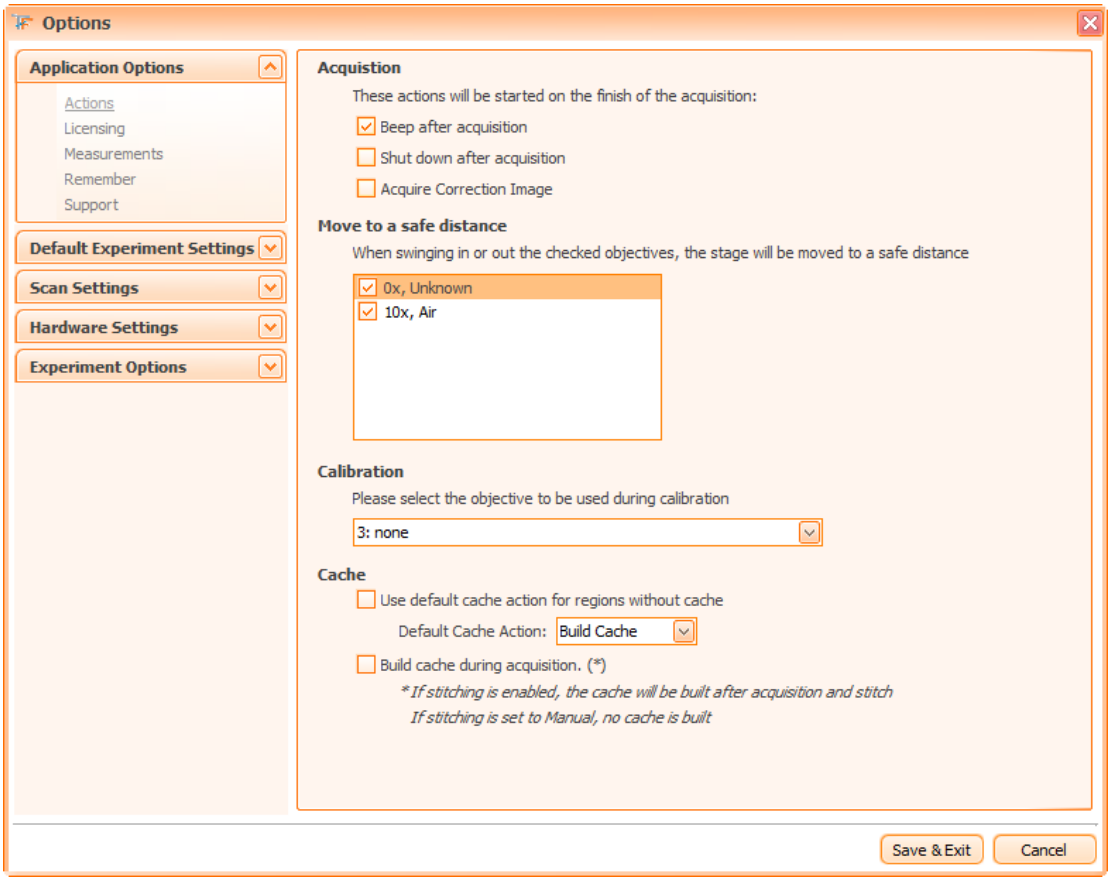


Figure 7 - Actions dialog

Acquisition

After finishing the acquisition, three actions can be performed:

- **Beep after acquisition**: **TissueFAXS** will announce the finish of the acquisition process by repeated beep sounds;
- **Shut down after acquisition**: the computer will automatically shut down after acquisition.
- **Acquire Correction Image**: before the acquisition process, a correction image will be acquired.

Safe distance

Safe distance means the Z-position to which the stage is lowered in order to ensure that no objective lens collides with the slides or any part of the stage.

Safe distance is used in the following situations:

- calibration of the stage;
- when moving between slides.

- By default, for any objective with magnification higher than 20x, **TissueFAXS** will lower the stage to the safe distance. For the rest of the objectives, you may customize this behavior in the dialog shown below. Usually, you will prefer to enable this behavior for any longer objective or for objectives that come very close to the slide for focus. For inverted microscopes, the best action is to enable it for all objectives.

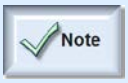
Calibration

This is a safety setting and you should access it after installing **TissueFAXS** in order to choose a safe objective for calibration. This choice means that the application is helping you avoid possible damages of the objectives, stage or slides, caused by an inappropriate objective being used during the calibration procedure.

If an objective with a magnification higher than 20x will be selected for calibration, every time before performing calibration **TissueFAXS** will send you a warning message (please see **Chapter 3.3**).

Cache

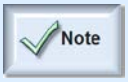
The image cache consists of scale down acquired images and it is used to speed up the display of big regions and decrease the memory requirements for this operation. If no cache is built, you will be able to see only low resolution images.



Other operations like export and analysis are not affected in any way by the presence or lack of the cache.

The cache can be built:

- During acquisition, if **Tools → Options → Actions → Build cache during acquisition** is checked.



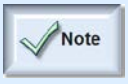
-This will increase acquisition time; hence **TissueFAXS** will build the cache after the acquisition and the stitching are done (if stitching option is enabled).

-If the stitching or the cache is performed at the end of the acquisition process, a progress bar will warn the user about the status of the action. The progress bar can be found in the status bar of the application.

-If stitching is set to **Manual**, no cache is built.

Figure 8 - Build cache after acquisition when stitch option is enabled - Progress bar

- When opening a region, if **Tools → Options → Actions → Use default cache action for regions without cache** is checked and **Build Cache** is set for **Default Cache** actions**.**



If **Use default cache action for regions without cache** is not checked, **TissueFAXS** will ask every time when opening a region without cache if you wish to build it.

- Using the region contextual menu **Build Cache for Region […]**.

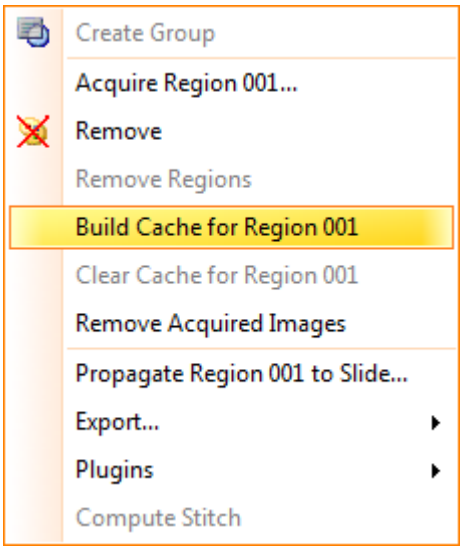


Figure 9 - Build cache feature in region contextual menu

4.1.2 Licensing

In the **Licensing** dialog the user will renew the license for **TissueFAXS** by following these steps:

- use **Save license file…** button in order to save the license file on your hard drive;
- send this file to your local TissueGnostics distributor to be renewed;
- use **Load license file…** to reload the new license file to the application.

- Use the **Licensing** feature ONLY if you are instructed by TissueGnostics personnel or authorized distributors.
- **Licensing** feature is only available if logged as technician or administrator.

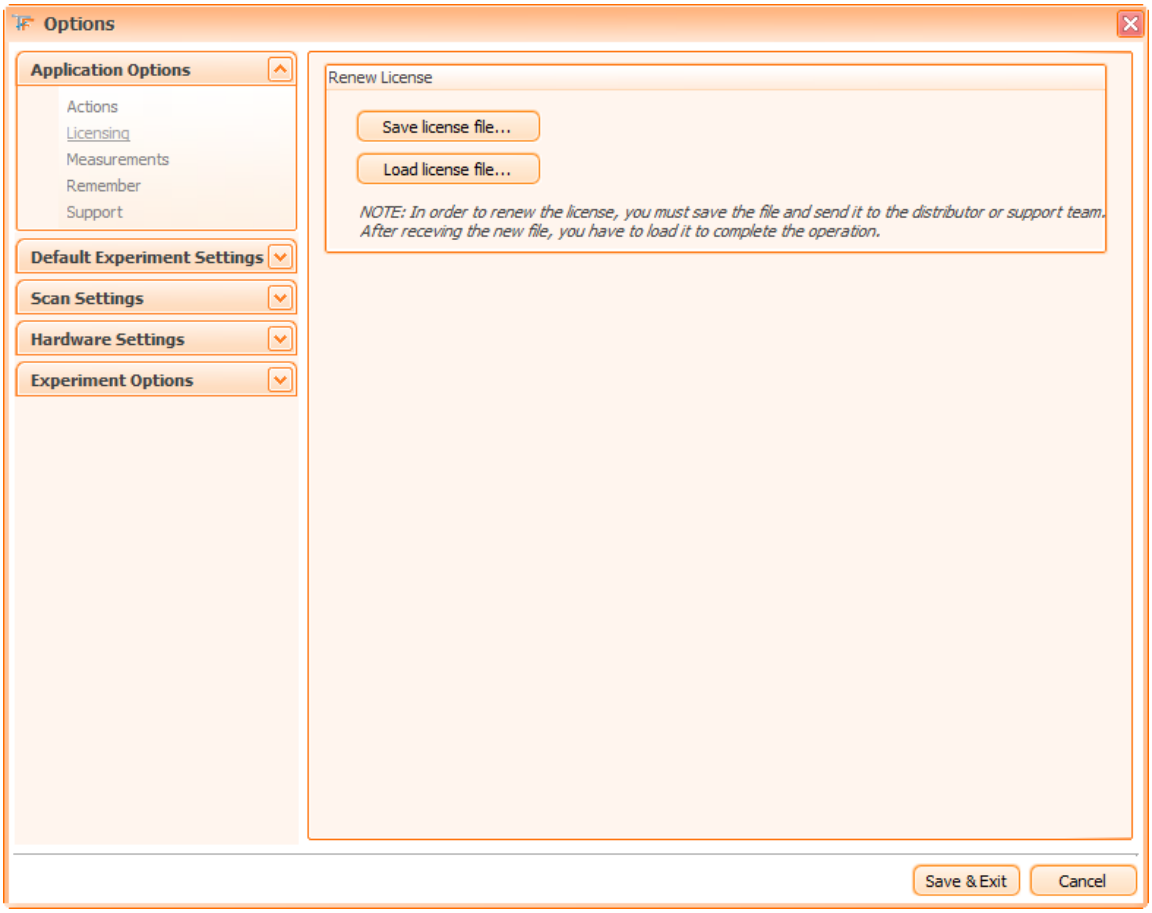


Figure 10 - Licensing dialog

4.1.3 Measurements

In the **Measurements** dialog you can manage the display options for the scale bar and measure function.

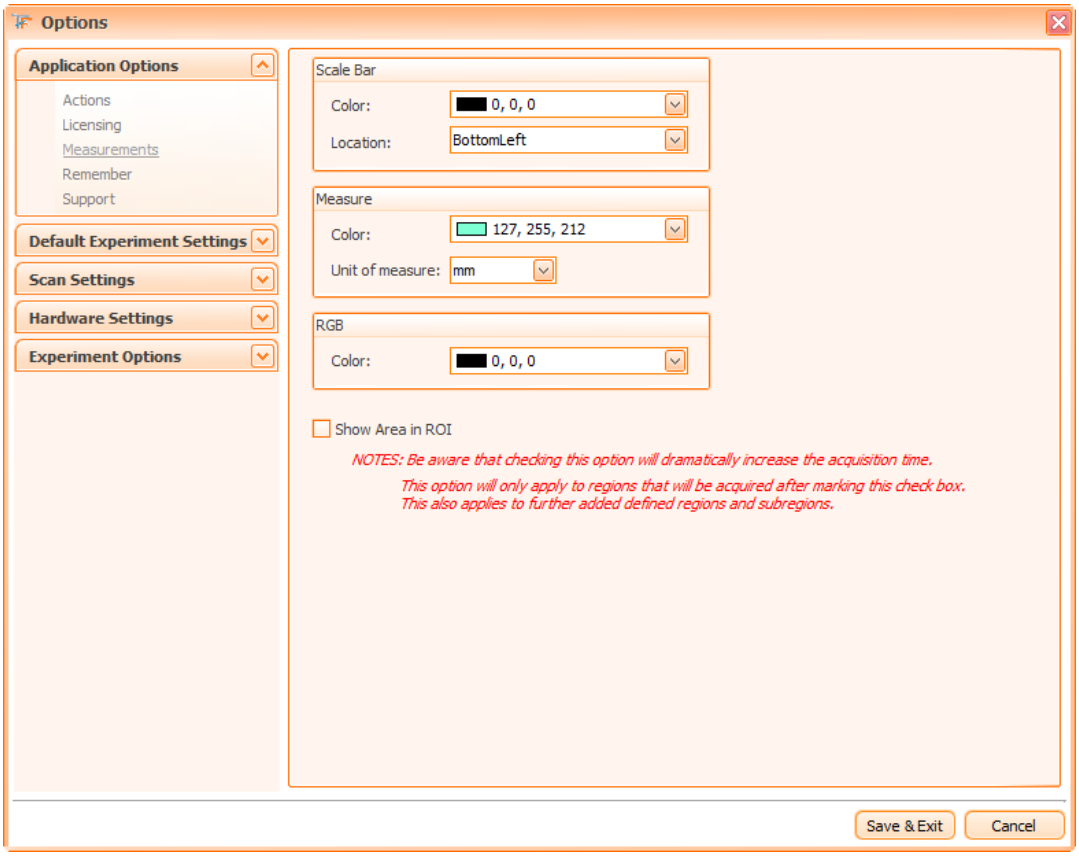


Figure 11 - Measurements dialog

Scale bar

The **Scale bar** icon looks like this: .

The **Scale bar** is represented by a segment that indicates the scale of the image.

It has two adjustable attributes:

- The **color**;
- The **location** list with four values:

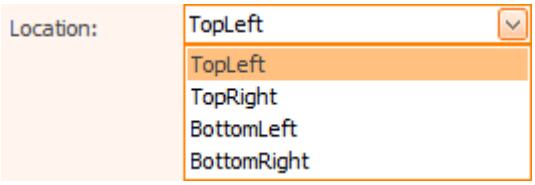
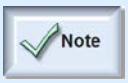


Figure 12 - Scale bar location combo box



- The default values are **Black** (for color) and **BottomLeft** (for location).

The scale bar can be found on:

- Live Image;
- Slide Preview;
- Region Viewer;

- One Image Viewer;
- Exported Images.

Measure

The **Measure** icon looks like this: .

This function is used to measure the distance between two points (on the sample) specified by the user (by clicking on the start point, then on the end point).

The distance and the unit of measure are displayed on the measured image.

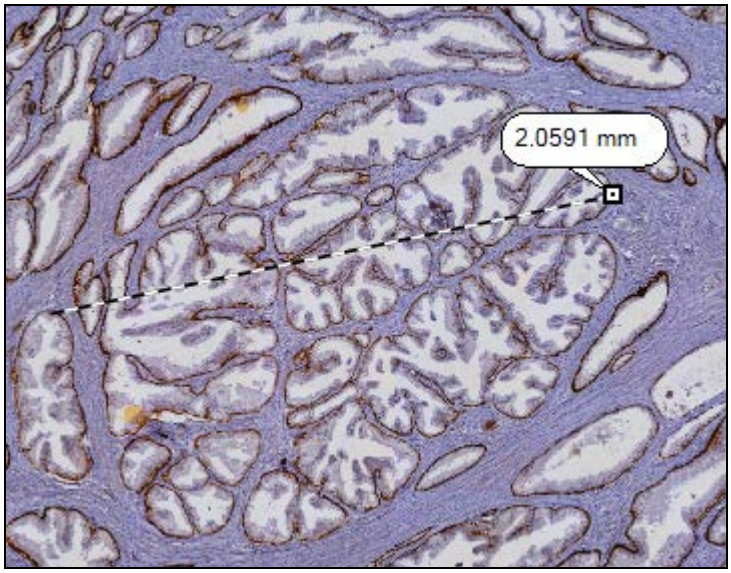
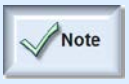


Figure 13 - Measured image example



- There are two adjustable attributes, the color and the measure unit. The default color is **aquamarine** and the default measure unit is **millimeter**.

This function is available on:

- Live Image;
- Slide Preview;
- Region Viewer;
- One Image Viewer.

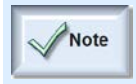
RGB

Here you can select the color used to display information about **RGB** or **Grey value** for a pixel on the camera live image.

Show Area in ROI

If checking this option, the defined regions and subregions that are going to be created will have computed the *area* and the *area in ROI*.

- The area refers to the total area of the region (including only acquired fields of view);
- The area in ROI refers to the area for the cropped shape (including only acquired fields of view).



- The **Area** and the **Area in ROI** measurements are also displayed in the Experiment editor (see **Chapter 5.14**).

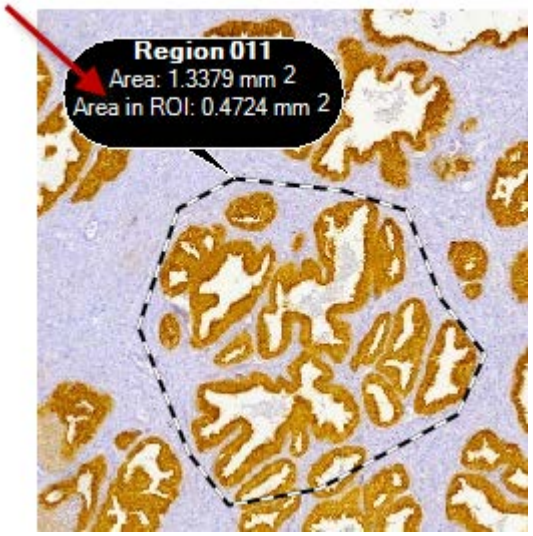
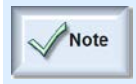


Figure 14 - Measurements: Area and Area in ROI



- Checking this option will dramatically increase the acquisition time.
- This option will only apply to regions that will be acquired after marking this checkbox. This also applies to further added defined regions and subregions.

4.1.4 Remember

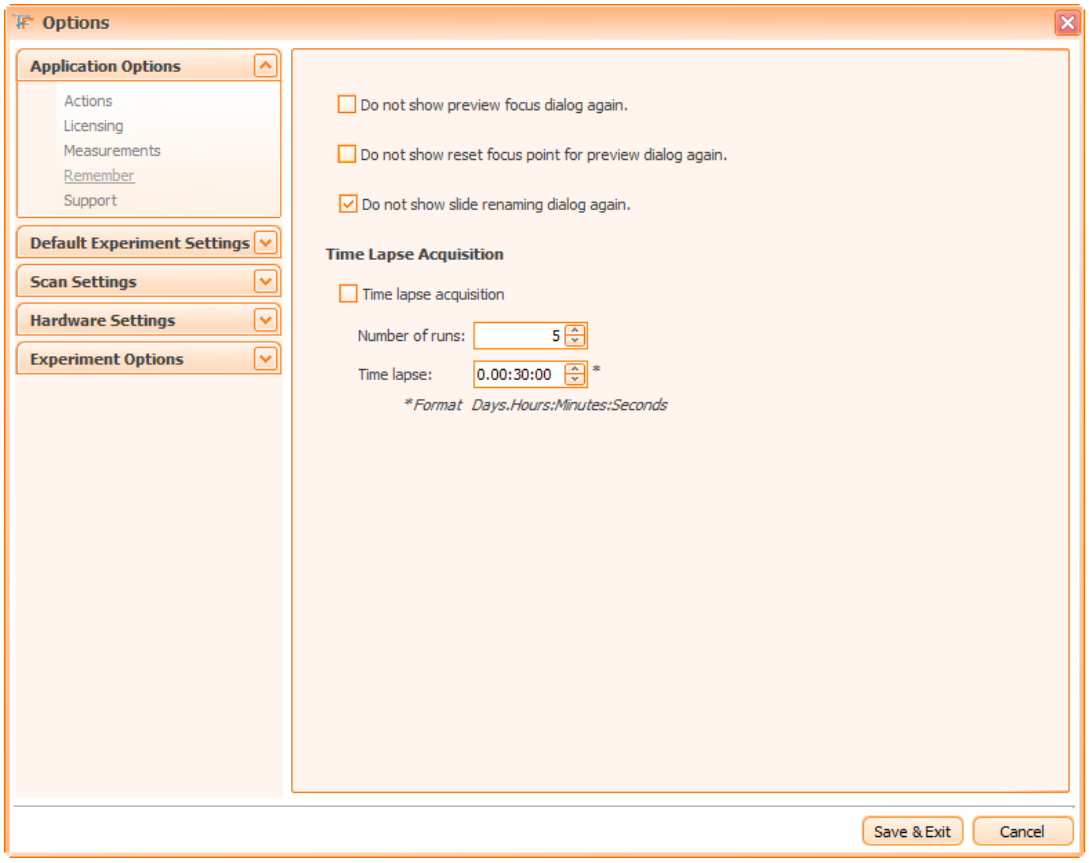


Figure 15 - Remember dialog

This dialog can be used to specify default values for some of the most commonly used options of the application.

- Uncheck **Do not show preview focus dialog again** if you want that dialog to be displayed before the preview operation; otherwise, check this option;
- Uncheck **Do not show reset focus point for preview dialog again** if you want that dialog to be displayed when you reset the preview focus points; otherwise, check this option.
- **Do not show slide renaming dialog again**: this option (checked by default) will prevent the slide renaming dialog to appear each time you create a new experiment.
- **Time Lapse Acquisition**: check this option in order to set by default multiple acquisitions for a region or a group of regions. You must set desired values for the following parameters:
  - *Number of runs*: how many times the acquisition will run;
  - *Time lapse*: the interval between runs.

4.1.5 Support

When using **TissueFAXS**, as in any other software application, you might occasionally encounter different kinds of errors, or you might simply need support and answers to your **TissueFAXS** related questions. The **Support** section was designed to help you in these situations.

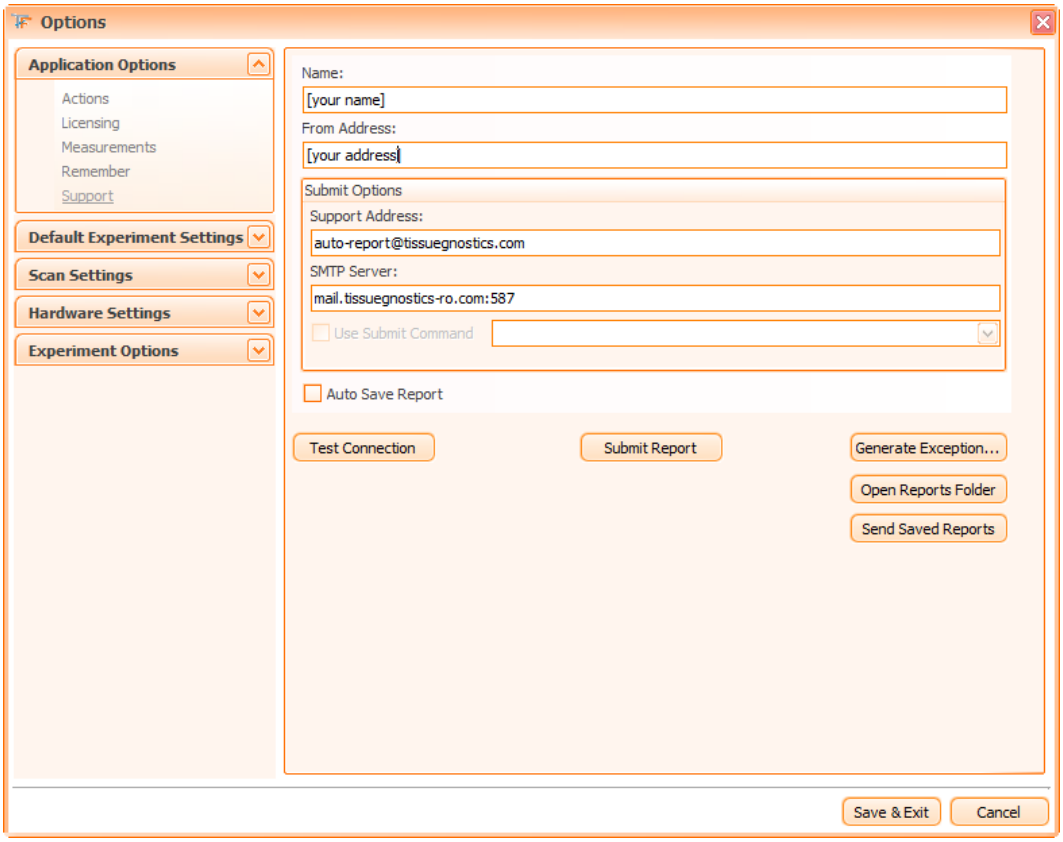


Figure 16 - Support dialog

Enter the following **information for dynamic error reporting**:

- The real name of the user who bought the software (e.g.: institution name);
- The email address that will be set as sender;
- The email address of Tissue Gnostics support;
- The available SMTP server/port to be used when sending the error report. (Contact your network administrator for details).

Use Submit Command: check this in order to access an external executable file that will send the files to a server.

Auto Save Report: check this option in order to save the log files on the hard drive, no matter if the report is sent or not.

Test Connection is used to see if the connection to SMTP server works.

Submit Report will help you send a mail containing your possible questions or problems regarding **TissueFAXS**. Beside the information you type in the form, the email automatically includes the log files, the running processes, the configuration files of the application. To effectively send the mail, press the **Send Report** button.

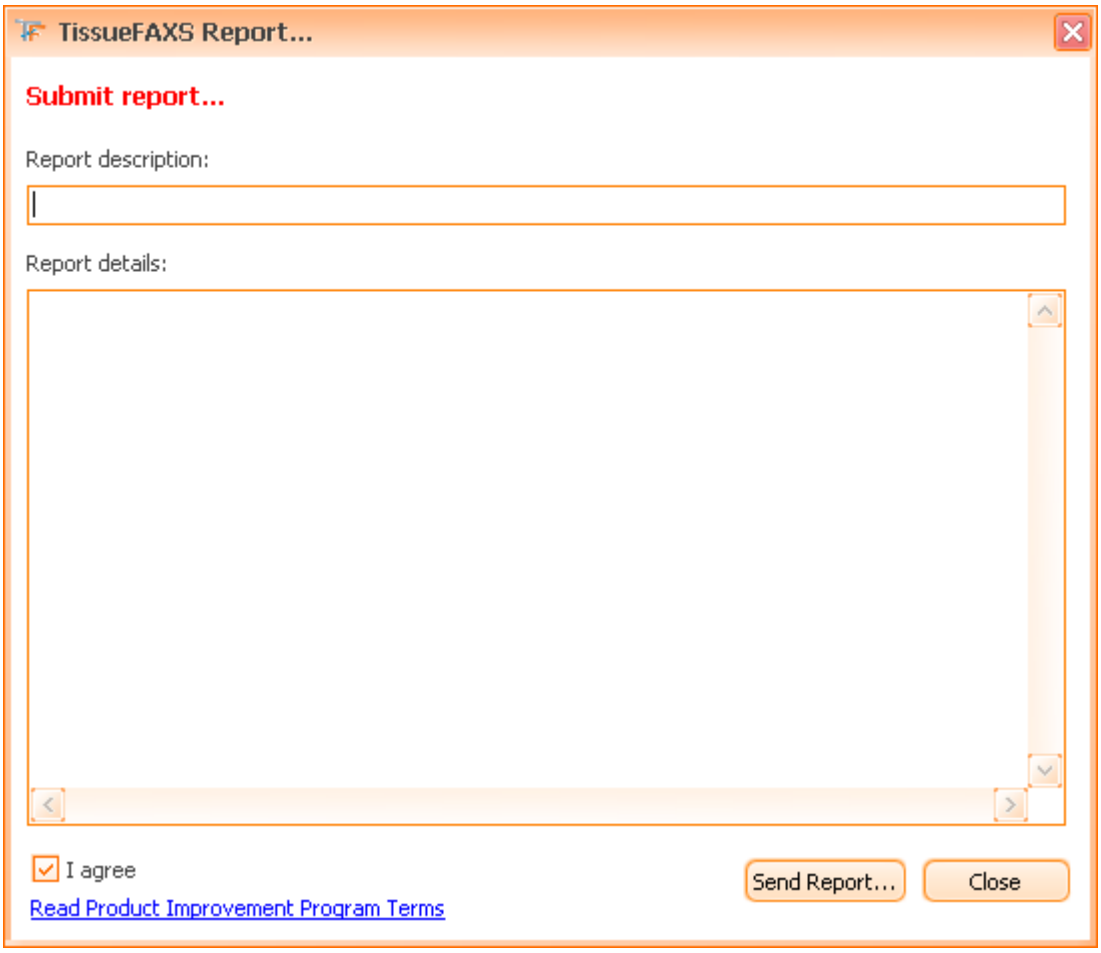


Figure 17 - Submit Report dialog

Generate exception is used to simulate an error in order to test if sending emails with the specified settings works.

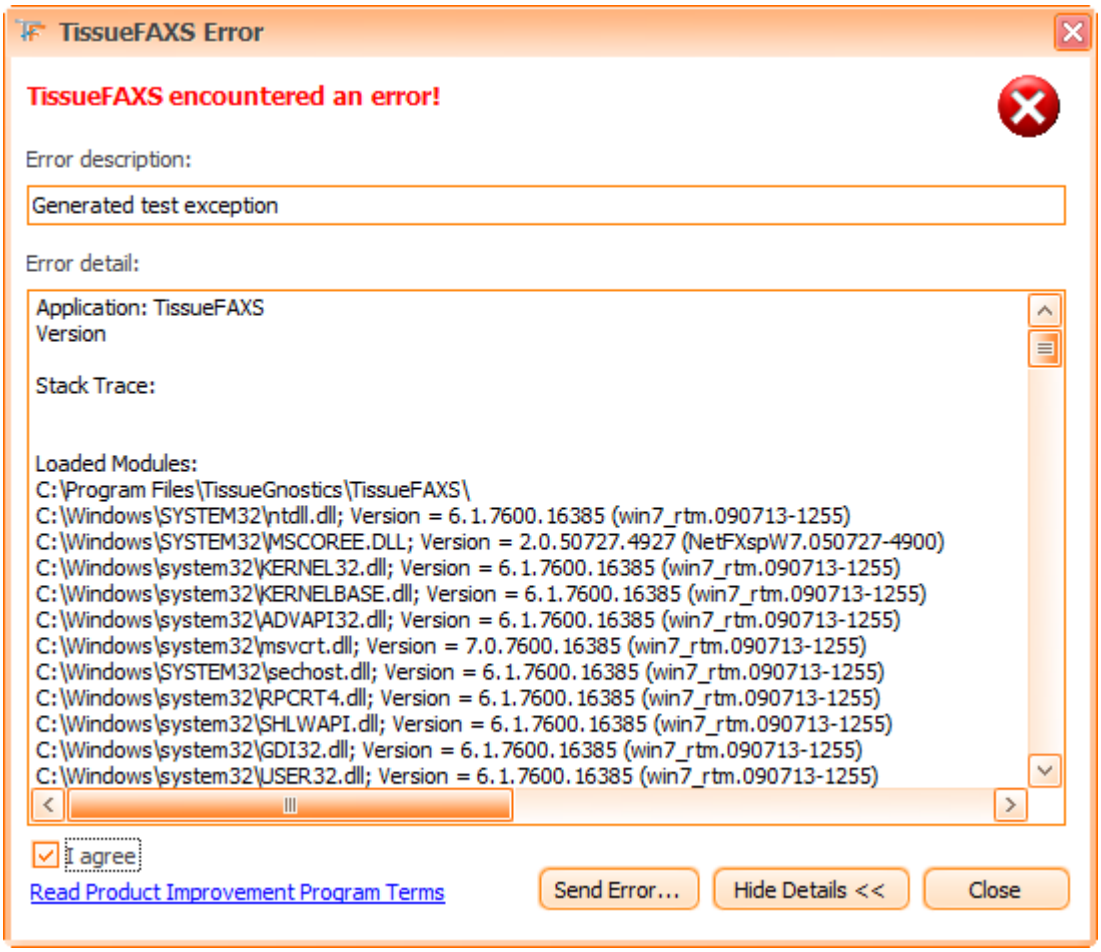


Figure 18 - TissueFAXS Error dialog

- **Error description**: here you can see a short description of the error.
- **Error detail**: here you can find detailed data related to the error.

Open Reports Folder: if a report is not sent, it will be automatically stored in a local folder; press **Open Reports Folder** to open that folder.

Send Saved Reports: press this button to send all unsent reports, if any. After effectively sending saved reports, they will be automatically deleted from the local folder where they were stored.

Product Improvement Program

Product Improvement Program checkbox: In the **Product Improvement Program**, participating systems send information to TissueGnostics about how they use certain products. Received data is combined to help TissueGnostics solve problems and to improve the products and their features. Click the link in the lower-left corner of the dialog shown above to read about this program and check **I agree** checkbox if you want to participate. If you don't check the check-box, the **Send Error** button will be disabled.

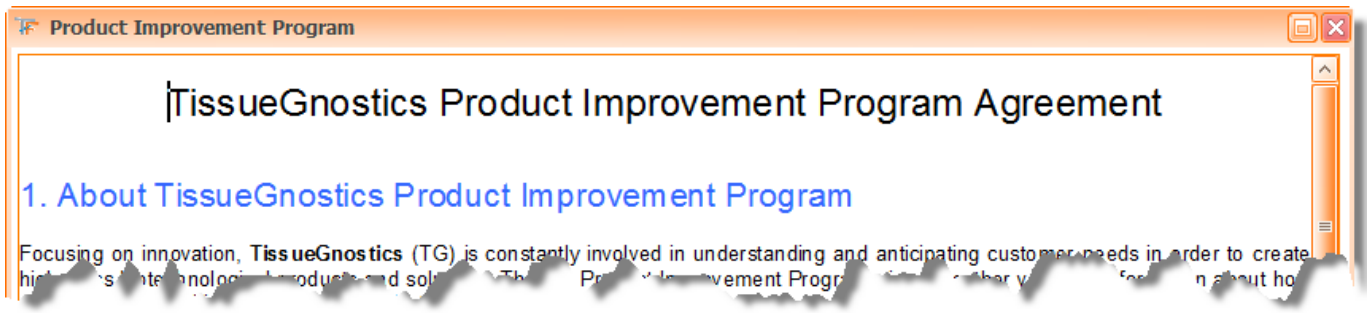


Figure 19 - Product Improvement Program Agreement

4.2. Default Experiment Settings

4.2.1 Preview

In the preview dialog you can manage the stored preview schemes. The adjustable yellow area allows the user to define the default preview region on the slide(s).

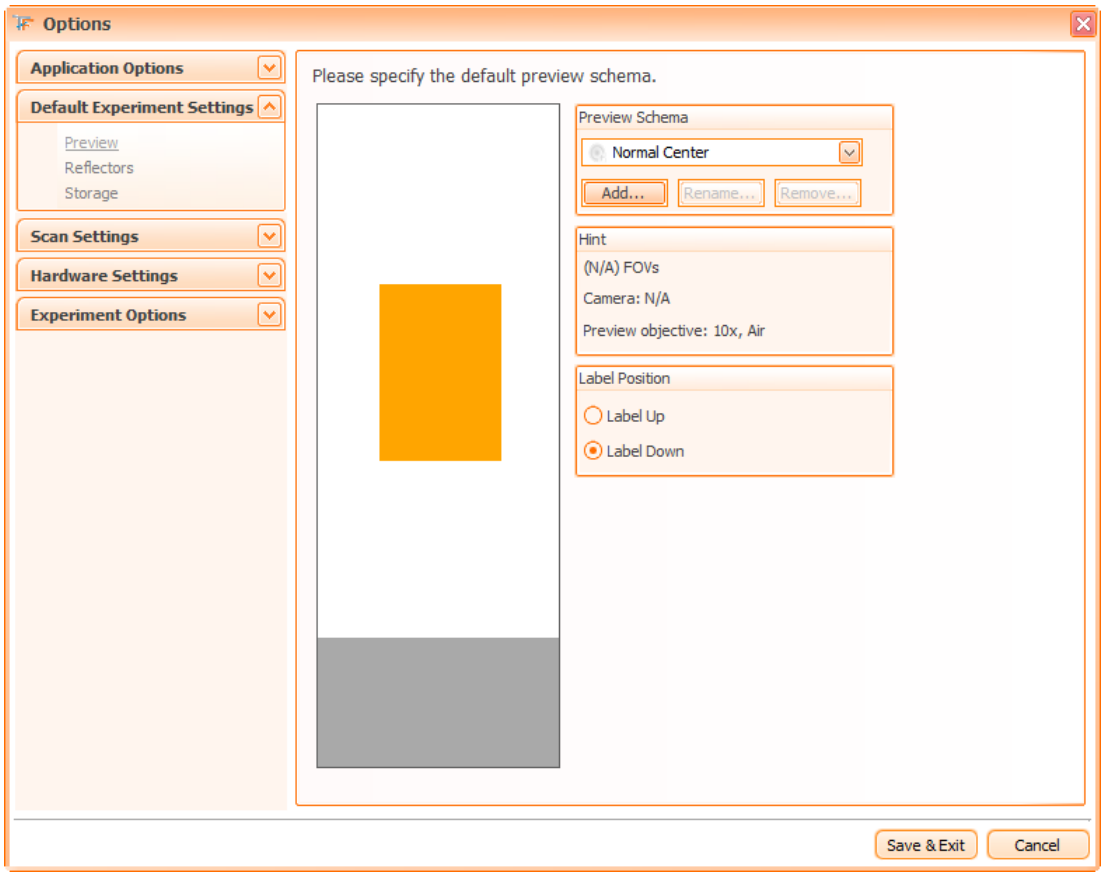
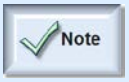


Figure 20 - Preview dialog

- By default, four **preview schemes** (**All Slide**, **Large Center**, **Normal Center**, **Small Center**) are present, which cannot be deleted or modified. These schemes are grey. You can add, rename or delete additional schemes from this panel.

- Create new preview area on slides: press **Add…** button to create a new preview schema according to the size and the location of the sample you are currently using. After giving a name to the newly created preview area, you can modify its size (resize using the mouse cursor on any edge of the shape) or move it (by using the mouse cursor to drag&drop the area to the desired location);
- Set the default preview area on slides by selecting the desired entry from the **Preview Schema** combo box.

- Select the label position from the **Label Position** section (default label position is **down**).

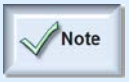


- The options mentioned here will only affect newly created projects. Please remember the label position is not stored in the preview schema.
- Changes in this panel will affect future experiments. If you want to change the preview area of a current experiment, use the **Preview** option from the **Experiment Options** section (in the same dialog).

- More detailed information concerning the **Preview** option can be found in the **Hint** section (**Chapter 5.1.4**): number of FOVs, camera used for preview, preview objective.

4.2.2 Reflectors

Fluorescence images are usually gray scale images (from black to white over a certain number of gray values), the intensity of the gray values representing a certain marker staining intensity. In order to acquire a marker, a corresponding reflector (filter block) must be used. When using multiple markers, in order to get a better overview for the entire region, **TissueFAXS** allows you overlaying images acquired with different reflectors. You can choose for each reflector (color channel) the optimal color (Look-Up Table) that will represent the corresponding marker in the final image.



Later on we will use the following terms:

- A *Region Overlay* is the image created by superimposing images of the same region acquired with different reflectors.
- A *channel* is the image of a region acquired with a certain reflector.

The user has also the option to change the names of the reflectors by going to **Tools** → **Options** → **Reflectors**.

The colors of the reflectors will be used as default for new experiments only and after acquisition each region can have its own color configuration.

You can edit these default colors according to your needs in the dialog below (see also **Chapter 5.5.2**):

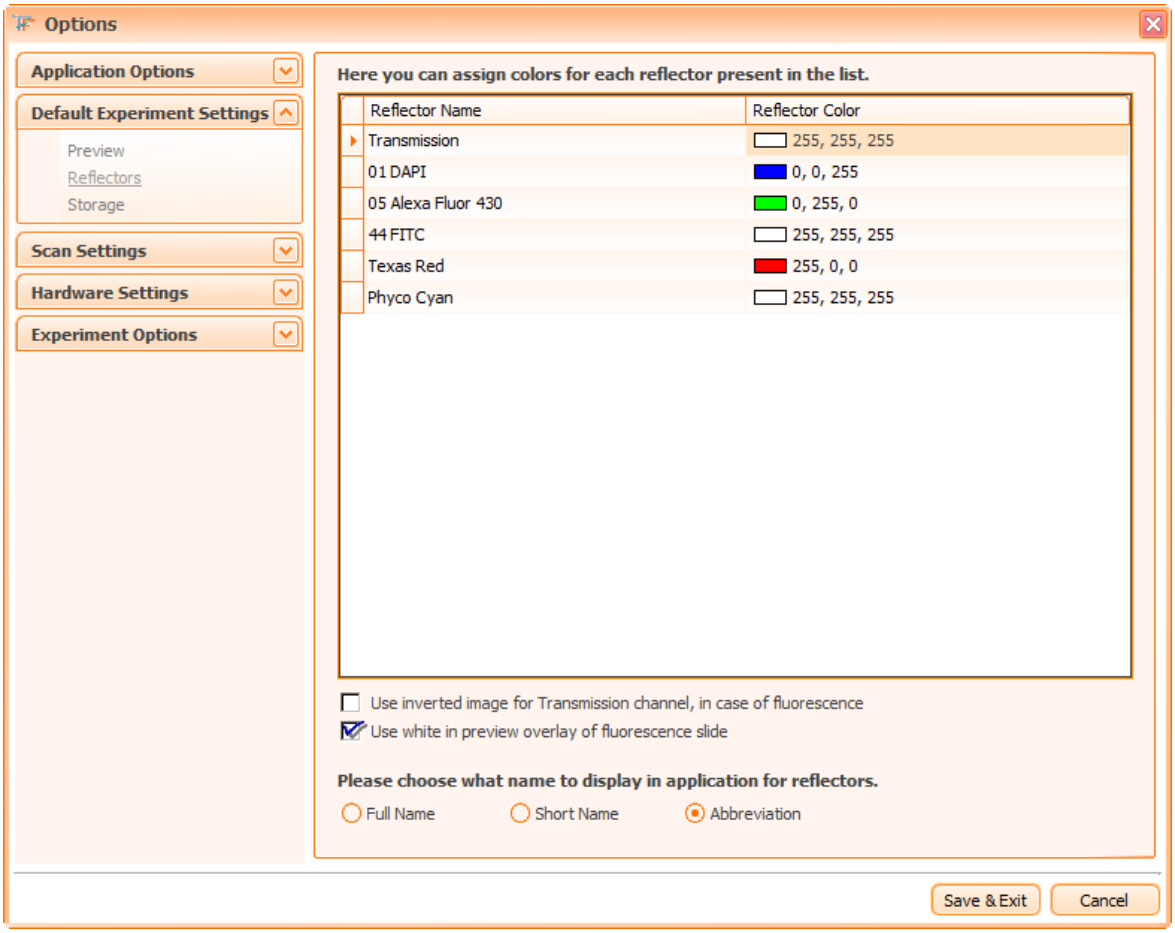


Figure 21 - Reflectors dialog

- **Use inverted image for Transmission channel, in case of fluorescence experiment**: choose how the transmission image will appear in the overlay image - the normal image or the inverted image of the Transmission channel (which means a ‘‘fluorescence-like’’ appearance);
- **Use white in preview overlay of fluorescence slide**: for fluorescence experiments, if only one channel is present in the preview settings, the default color for that channel is white. If the preview is made on a nuclear marker like DAPI and is displayed in dark blue, the resulting preview image will be difficult to see against the black background. White, however, gives a stronger contrast.
- Finally, you can choose how the **name** of the reflector will be displayed in the application: **Full name**, **Short name** or **Abbreviation** (see also **Chapter 5.5.2**).

4.2.3 Storage

In the **storage** section you can define various data for the file to be stored, like the file **names convention**, the file **types** and the default **locations**.

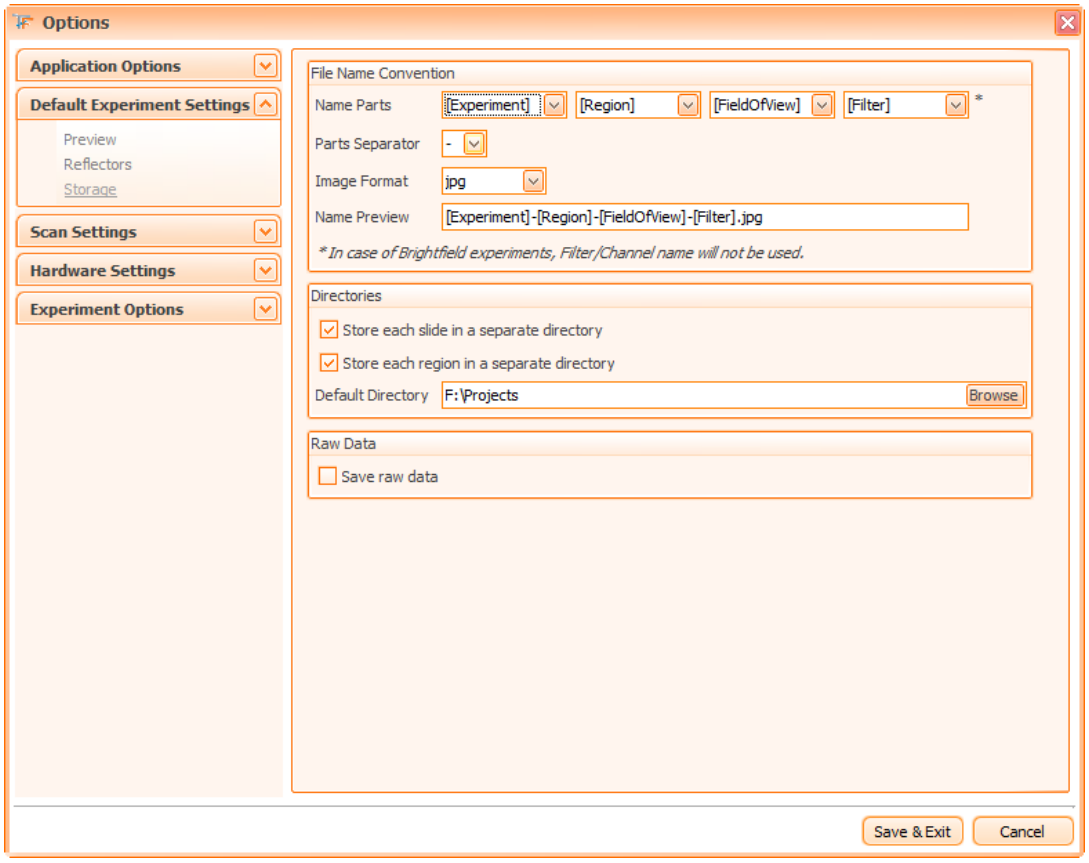


Figure 22 - Storage Settings dialog

- **Name Parts**: select the parts that will generate the filename ([*Experiment*], [*Region*], [*Field Of View*], [*Filter*]);
- **Parts Separator**: select the separator between the parts of the filename;
- **Image Format**: select the image file format (e.g.: *.jpg* for small file size and good quality, *.bmp* for large file size and high quality);
- **Store each slide in a separate directory**: select how to organize image files in directories;
- **Store each region in a separate directory**: select the default directory in which the projects will be stored;
- **Raw Data**: check **Save raw data** if you want to save the raw data image corresponding to your file.

Note

- **Important:** Avoid storing projects on Desktop, My Documents, or in any other folder located on the system drive (usually C). If the drive is nearly at its full capacity, the system might behave erratically or even stop working if the drive completely runs out of space. Usually, systems delivered by TissueGnostics have a dedicated storage medium for projects; it is advisable to use it in order to increase TissueGnostics products performance and avoid issues with low disk space. Also in case the system needs to be reinstalled (virus infection, system files being deleted) you might lose data located on the system drive.
- Images stored as *.jpg* will make subsequent analysis slow because *.jpg* files are scanned by anti-virus software by default. You may want to consult your local system administrator on how to exclude *.jpg* files from “on access” scans. If this is not possible, we recommend storing files as *.tif* and compressing the folders after analysis is completed.

4.3. Scan Settings

4.3.1 Scan Strategy

TissueFAXS provides a type of **Scan Strategy** (**Snake**) that allows an optimal scanning process.

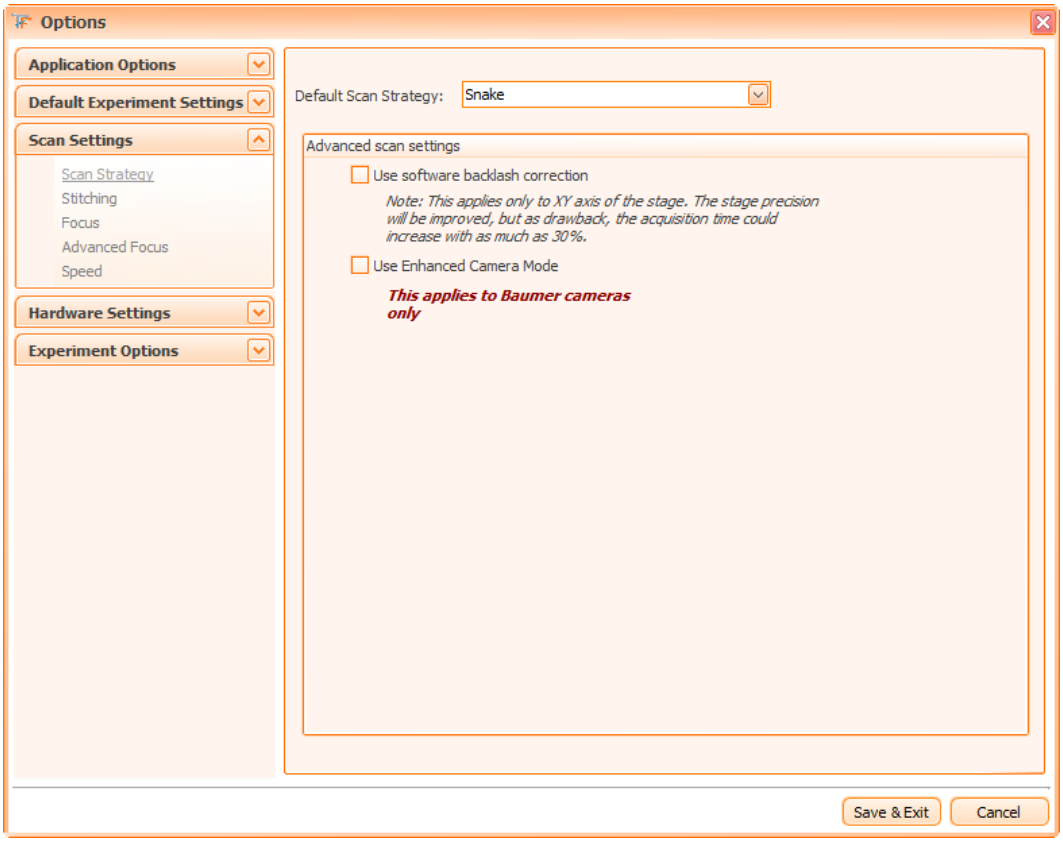


Figure 23 - Scan Strategy dialog

- **Use software backlash correction**: This is a feature that helps avoiding certain errors that may appear when changing the moving direction of the stage (caused by physical caracteristics of the stage), which is the case during normal **TissueFAXS** operation. It applies only to XY axis of the stage. The stage precision will be improved but, as drawback, the acquisition time could increase with as much as 30%. The backlash correction should be used when the acquired images do not properly fit to each other. **Note:** If all the images have a stitch problem, you should check the FOV calibration settings.
- **Use Enhanced Camera Mode**: this is a feature available on few cameras that will increase data transfer speed.

4.3.2 Stitching

Stitching will allow the user to see high quality pictures without any inconveniences caused by stage errors, camera calibration or other issues that might interfere with proper image display/acquisition.

To enable this feature you must mark the **Enable Stitch** checkbox.

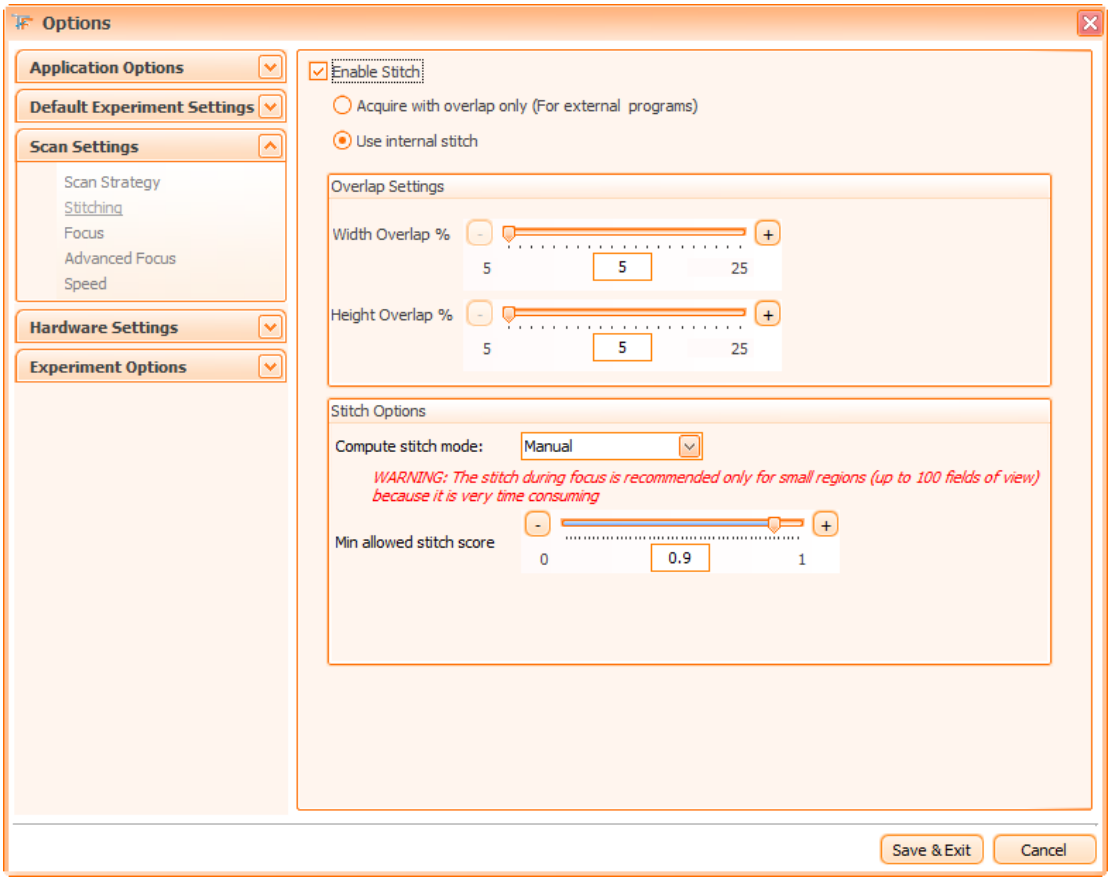


Figure 24 - Stitching dialog

You can choose between internal and external stitch:

Acquire with overlap only (For external programs): The acquired images will share common parts with their neigboring images. Use this option only if you want to stitch the images using third party stitching software. For normal use of the **TissueFAXS** software this is not recommended. Be also aware that regions acquired with overlap can't be properly handled by **HistoQuest**.

Use internal stitch: **TissueFAXS** will determine, based on the settings from **Stitch options** (see below), how to stitch the FOVs in order to obtain region overview without any discontinuities. **HistoQuest** will be able to properly handle this kind of regions.

Overlap settings: the external and internal stitching will properly work if the regions are acquired with an overlap that will allow compensating hardware errors. It is recommended using a value of at least 15 %. Avoid acquiring regions with large empty areas when using stitching.

Width/Height Overlap: are used to specify the percentage of width/height that will overlap neighboring FOVs.

Stitch options

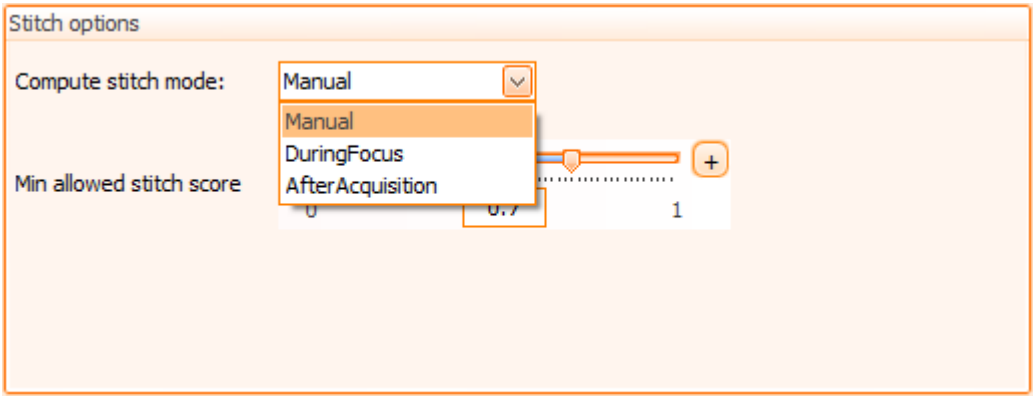
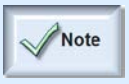


Figure 25 - Stitch options

Compute stitch mode:

- **Manual**: speeds up the acquisition process. However, the user will have to manually call the **Compute Stitch** menu.
- **During Focus**: slows down the acquisition process; the user will be able to see the acquisition results in real time.
- **After Acquisition**: this option will call the stitch after each region has been acquired.



- If **Compute Stitch** mode is activated, the cache will be erased.

- The stitch during focus is recommended only for small regions (up to 100 FOVs) because it is very time consuming.

Min allowed stitch score: decrease this value only if you are acquiring regions with a large empty area. In any other cases you should let it to the default value.

4.3.3 Focus

In this section you can adjust all the focus relevant parameters, organized in two tabs: **Focus Options** and **Enhanced Focus**.

Focus Options tab

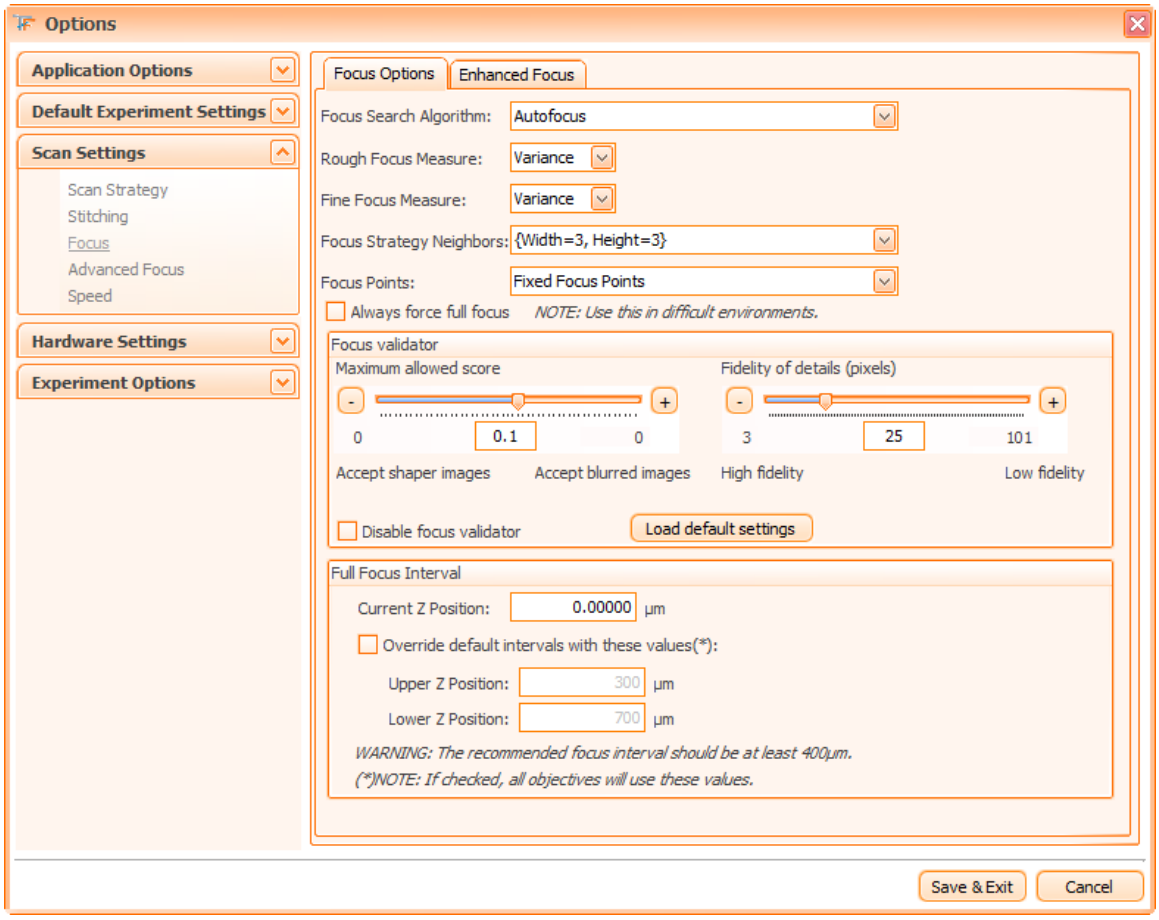


Figure 26 - Focus Options tab

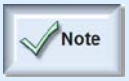
Focus Search Algorithm

- **Manual focus**: when using this type of focus, the user will be prompted to manually focus the image when necessary.
- **Current position**: no type of focus will be performed and the stage will maintain for all FOVs the same position during acquisition.
- **Autofocus**: when using this type of focus, **TissueFAXS** will automatically detect the best focus position, based on the parameters you can find described below.

The **Autofocus** method consists of two steps. In step one, using a fast method, **TissueFAXS** tries to determine a short interval where the focus is best. In step two, using a high precision method (specified by the **Fine Focus Measure** parameter), **TissueFAXS** determines the best focus position from the fine focus interval determined in step one.

Generally, in order to determine the fine focus interval, **TissueFAXS** uses the **Rough Focus Measure** and scans the entire interval specified in the "**Focus interval**" section. This approach is called "**Full Focus**".

However, in order to improve the speed of the focus, if a previous focus position was found, **TissueFAXS** will directly use the **Fine Focus** on a small interval around the last focus position. This approach is called "**Quick Focus**".



- If the **Quick Focus** method fails, a **Full Focus** will be automatically performed.

The following parameters are for autofocus only and have no effect on the **Current position** or **Manual Focus**.

Rough Focus Measure and Fine Focus Measure

For both **Rough Focus** and **Fine Focus** steps, three **focus measure algorithms** are available:

- **Variance**: this is the fastest and most robust method for finding the location of the sample. It has the drawback of being easily affected by the quality of the samples (e.g. any dirt, bubbles of air in the oil – if any, illumination problems or any other optical artifacts). **This method is the default choice for Rough Focus measure because its robustness and speed.**
- **HP**: this method is more precise than **Variance** at the cost of being more time consuming. It has two additional parameters, X% and Y%, which specify how much from the image is processed. By decreasing these two values the speed of the HP will be increased, at the cost of lowering the chance to focus on sample (e.g. analyzing a hole of the tissue cannot provide the Z position due to lack of signal). Usually, a value of 50% or above should provide a good balance between speed and accuracy for objectives up to 63x. For higher objectives, it is recommended to have most of the image content analyzed. **This method is the default choice for Fine Focus measure**.
- **HP Fine:** this method is the slowest of all (more than two times slower than HP in terms of computing time), but it can provide the best focus under toughest conditions. It should be used only when HP fails repeatedly on difficult samples, for example on fluorescence samples with a high un-uniform background. This method is not recommended for **Rough Focus**. It has an additional parameter (if compared to HP): the **Resolution**. Its default value is 25 and is related to the size of the relevant objects in the image. Setting this value lower translates into higher focus sensitivity, also for small objects.

Focus Strategy Neighbors

This parameter can be adjusted according to the flatness of your tissue section, as the focus directly depends on the subsidence of the tissue. If the tissue section would have been perfectly flat, a single focus for its entire surface would have been enough. But more often, each tissue section has its own relief and the focus must be performed repeatedly. The higher the magnification will be, more the relief will influence the focus.

The **Focus Strategy Neighbors** helps you save time when performing the focus, by avoiding unnecessary focusing. The regions to be acquired will be split in blocks of Fields of View. The **Focus Strategy Neighbors** parameter defines how many FOVs will share the same focus estimation. In the actual example, 3x3 is selected, in which case **TissueFAXS** will focus once for every block of 3X3 fields of view. (The location of the focus point within the block is determined based on the Focus Points parameter). After focusing in one point, **TissueFAXS** will scan all neighboring FOVs using the same focus position.

- **Focus Points**:
  - **Fixed Focus Points**: the focus is performed on the FOV from the center of the block determined by the **Focus Strategy Neighbors**. **Fixed Focus Point** is the most frequently used in the acquisition process, when your tissue sample is compact (without holes, for example);
  - **Floating Focus Points**: the focus is performed in a point that may not be the center of the block. **Floating Focus Points** are especially used for biopsies or when your regions to be acquired are not compact (e.g. they include holes). The method identifies the location of the tissue within one block of FOVs and selects the most inner one. This location may be between several FOVs and not necessarily identical to one of FOVs.
- **Always force full focus**: The last focus position is ignored and the focus is always searched in the full interval. This method will take more time, but the obtained results will be better in case of difficult samples and environment (e.g. samples with many tissue folds).

Focus Validator

The **Focus Validator** checks and confirms that the focus search algorithm has obtained a good quality image.

It is used to confirm that the **Quick Focus** method has worked fine. If the quality score it provides is low, then a Full Focus search is initiated.

It is also used to confirm that the **Full Focus** method algorithm has obtained a sharp image. If this validation fails, the system will use the last known good focus position.

Normally, the default values for the validator options should be enough to provide a good acquisition.

- **Disable focus validator**: it will disable any focus validation and, as a consequence, any images returned by the focus algorithm will be accepted as being in focus. It should be used only as a last solution when the sample has a very low contrast.
- **Maximum allowed score**: increases the tolerance of the focus validator. Setting low values means that only very good images will be accepted as being in focus. Setting high values means accepting lower quality images (e.g. blurred). Basically, this parameter should be increased when using samples of a lower contrast.
- **Fidelity of details (pixels)**: size of relevant details. If you set it to minimum (3), then finest details are searched for and will make a difference in the validator score. If you set to a higher value, then only rough elements will count in computing the score. However, this depends on the images you are processing. Most of the time, the default value should provide good results.

The **Load default settings** button restores the default options only for the focus validator settings.

Focus Interval

In this dialog you can specify the z-boundaries of the rough focus-search. You can adjust your focus search interval to match extra thick or thin coverslips or culture plates. To determine the proper focus interval, we recommend manually focusing with the objective of interest. You can then check the current Z-position in the camera window and add 250 µm above and below this position for the upper and lower Z-positions of your interval. The current Z position should be read from **TissueFAXS** software (in Camera window) and not from any display of the microscope. Larger focus intervals will make the autofocus slow and may cause the microscope to focus on objects laying on the coverslip.

- If you want to use the specified focus interval for all objectives, check **Override default intervals with these values**;
- If you want to use the default focus interval only, uncheck **Override default intervals with these values**.

- Please be always very careful when making focus related changes, as wrong settings could cause damage to your sample and your objectives!

- For all the items described in **Focus** section, some **tool tips** are available. This means that when the mouse is over an item, a short description of the respective item will appear.

Enhanced Focus tab

The options from **Enhanced Focus** tab will increase the speed of the focus. However, they are available only on certain cameras.

Camera Partial Scan: based on width/height percent, only a part of the current FOV will be transferred from the camera to TissueFAXS, resulting in faster focus.

Enhanced Camera Mode: it will increase data transfer speed.

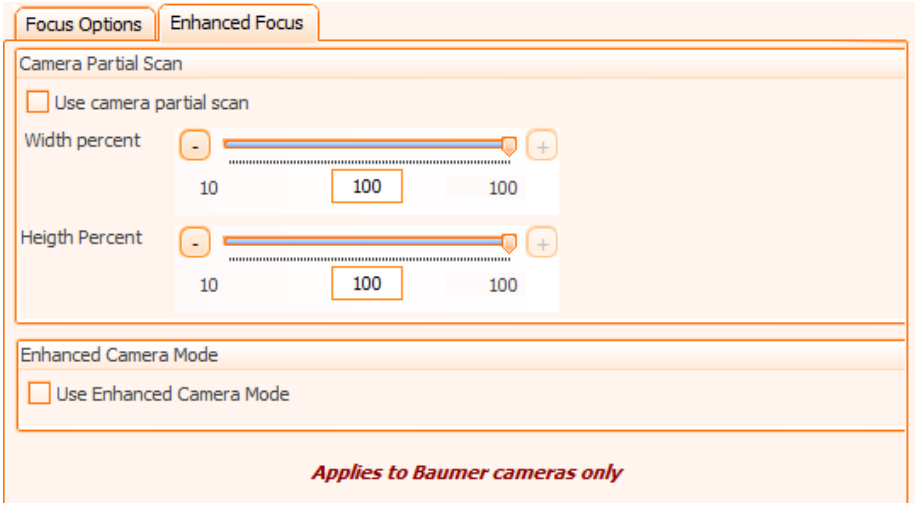


Figure 27 - Enhanced Focus tab

4.3.4 Advanced Focus

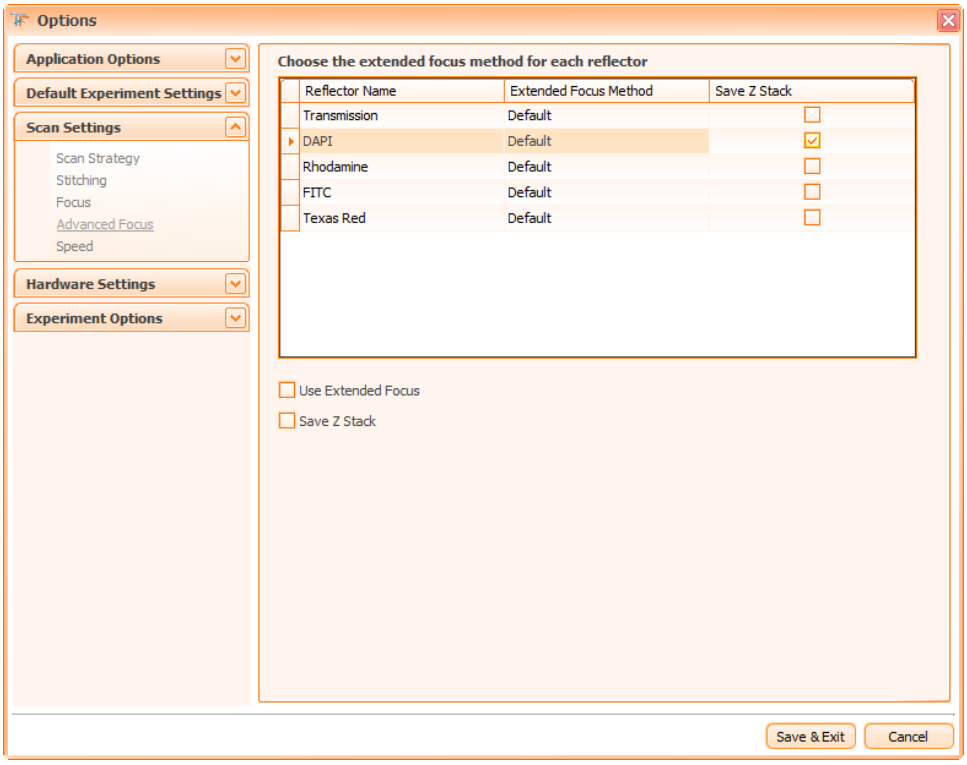


Figure 28 - Advanced Focus dialog

TissueFAXS software is shipped with one default extended focus method - **Default** - that works for all reflectors.

Z Stack

Z Stack represents a set of images that are acquired above and below the optimal focus position. By combining these images, a final FOV image will result, containing a higher level of details than any of the initial Z Stack.

Check this option only if you intend to use some external software to fuse the images. Be aware that enabling this option will require more space on the hard drive.

You can choose to use the Z stack for each individual reflector by marking the corresponding checkbox from the **Save Z Stack** column.

Note - To be able to use the Z stack for individual channels, you must previously check the **Save Z Stack** option below the reflectors' section.

Use Extended Focus

For each reflector present in the system, you can choose the **extended focus method** used to combine the Z Stack.

Check this option only when objects are present on multiple focus plans.

If you don't want to apply the extended focus to one or more reflectors, choose **None** option from the reflector's dropdown list.

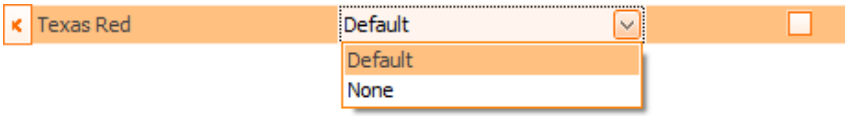


Figure 29 - Reflectors: Extended Focus dropdown list

Note - To be able to use the extended focus for individual channels, you must previously check the **Use Extended Focus** option below the reflectors' section.

CAUTION - Using any of the two options (**Use Extended Focus**, **Save Z Stack**) will significantly increase the acquisition time.

4.3.5 Speed

In the speed dialog box you can adjust the speed of stage movement during acquisition. These settings do not affect the speed settings of your joystick. Generally, all speed values can be set to maximum. However, if you are working with well plates or cell cultures in general, you may want to reduce these values (in order to avoid troubling the content).

The **Acceleration** parameter describes how fast the stage will try to reach the defined speed.

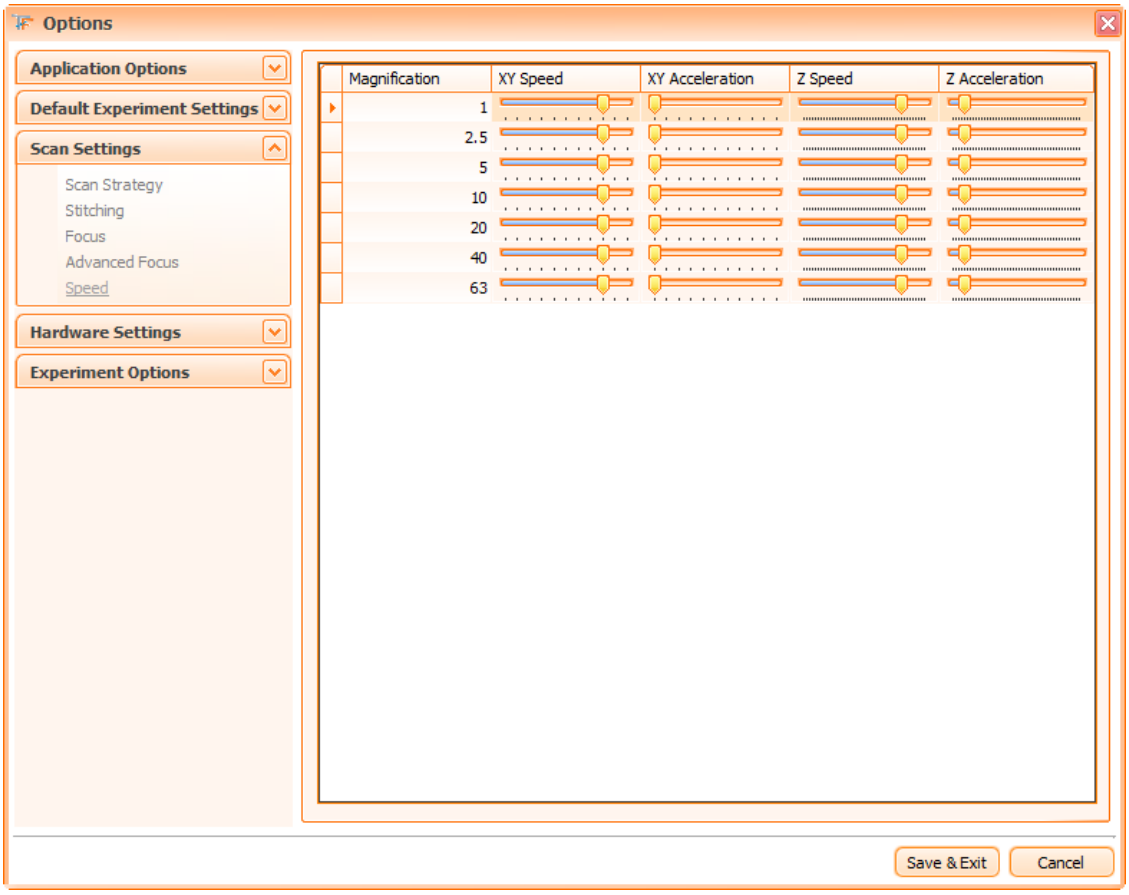


Figure 30 - Acquisition Speed dialog

4.4. Hardware Settings

4.4.1 Camera Manager

This option allows you to add and remove a camera from the list of the cameras connected to the computer. It also helps you setting the current camera. For more details please see **Chapter 5.7** of the current manual.

4.4.2 Contrast Manager

The **Contrast Manager** is an automated feature of the microscope that will automatically adapt the contrast method to the objective and filter combination (i.e. position of condenser, opened / closed shutters).

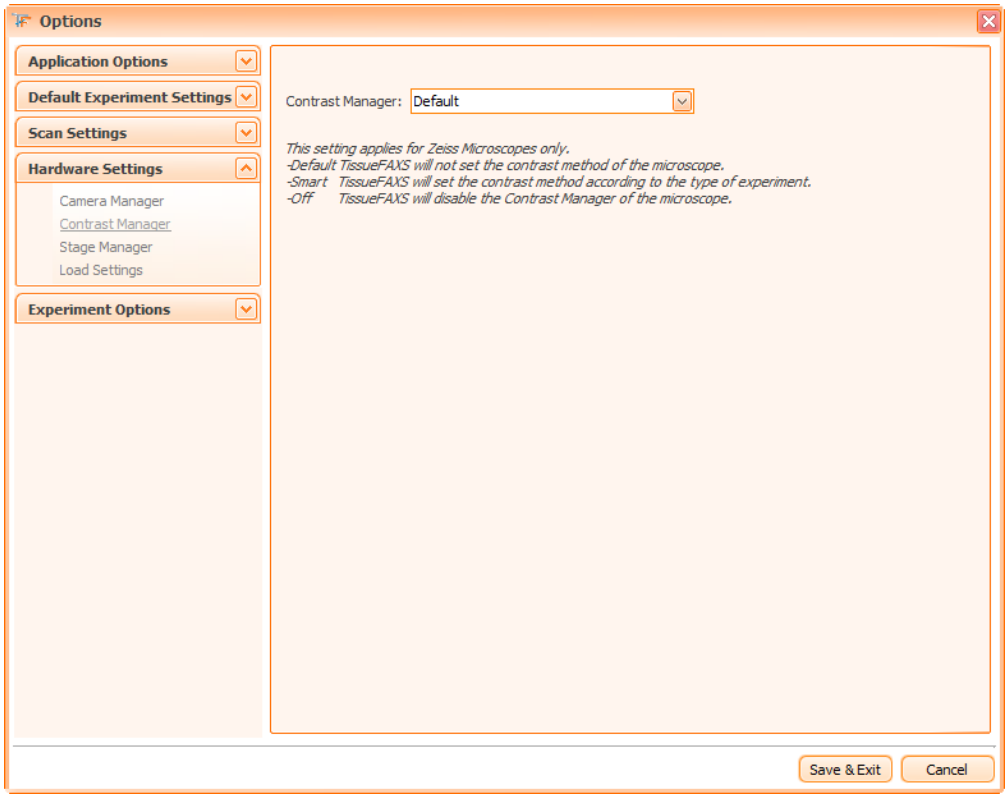


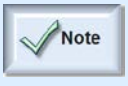
Figure 31 - Contrast Manager dialog

The **Contrast Manager** has three options available in the dropdown box shown below:

Figure 32 - Contrast Manager options

- **Default**: **TissueFAXS** won't try to set the contrast method of the microscope. For optimal results, we recommend manually setting the contrast method on the microscope.
- **Smart: TissueFAXS** will set the contrast method according to the type of experiment. This is the recommended setting for the Contrast Manager.
- **Off**: **TissueFAXS** will disable the Contrast Manager of the microscope. Use this option if **Default** and **Smart** options don't return desired results.

- If the contrast method is not available for the microscope, it will revert to brightfield.



- For a detailed view over contrast manager and contrast method, please consult your microscope operating manual.

4.4.3 Stage Manager

This option is useful only for inverted microscopes or for any other kind of system that can support different types of stage inserts (slides, well plates, flasks). You can choose here the layout of the stage (later referred as stage configuration) for the next experiment.

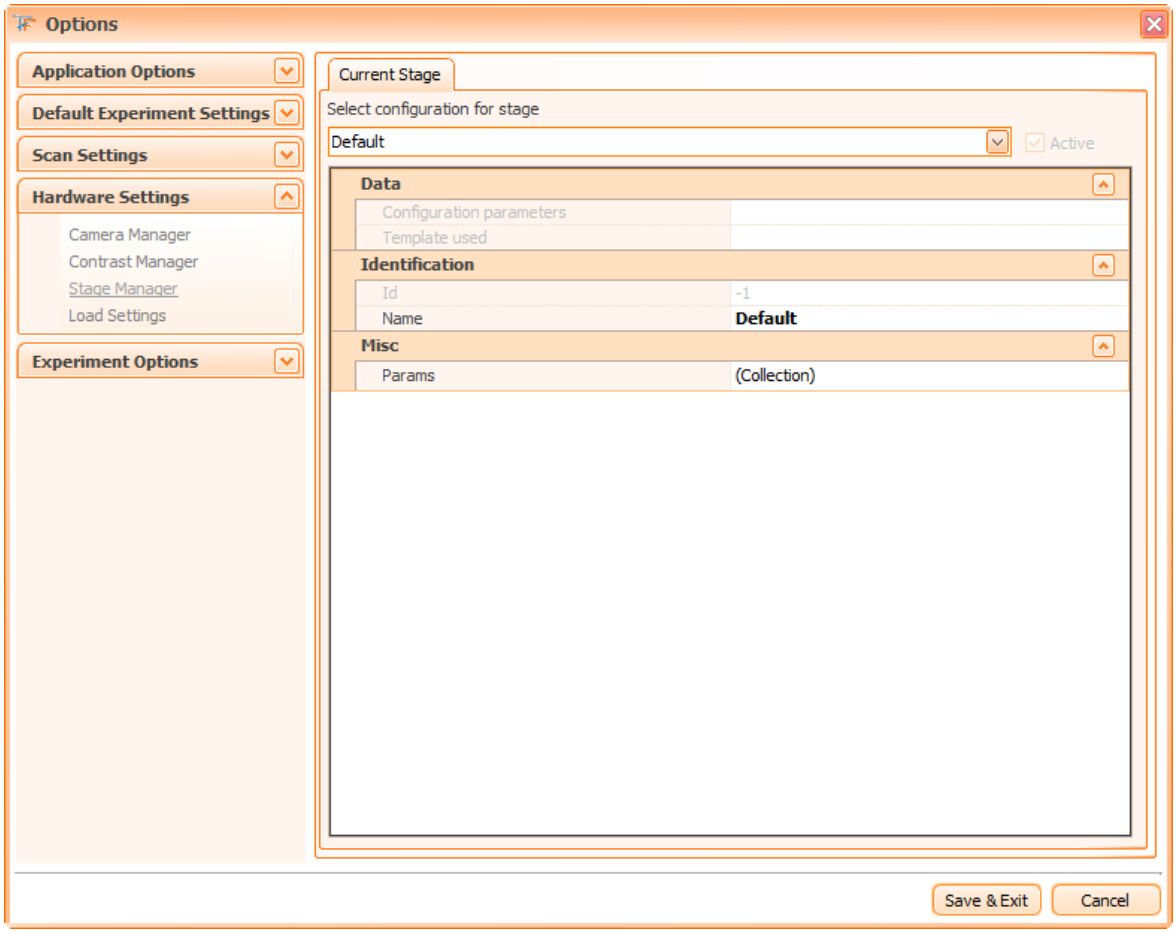


Figure 33 - Stage Manager dialog

TissueFAXS supports switching at runtime between different **stage configurations**. At this point, two base configurations are supported:

- **Slide inserts**: a **slide insert** is usually defined by the maximum number of slides it can hold, by slide dimensions and distance between two consecutive slides. This type of configuration can be used on an inverted microscope for culture flasks as well.
- **Well plate inserts**: a **well plate insert** is usually defined by the number of wells per row and column, distance between two consecutive wells and the distance from the edge of the plate till the first well.

Note

- **TissueFAXS** is configured by default with a slide insert configuration for 4 or 8 slides inserts.
- In order to control the defined slide inserts or to change the active one you must choose **Tools** → **Options** → **Stage Manager**.

Using stage configurations

Drop down box (Default) is used to select the desired insert configuration.

The area below contains information about the current configuration.

Example 1: The default configuration of the stage:

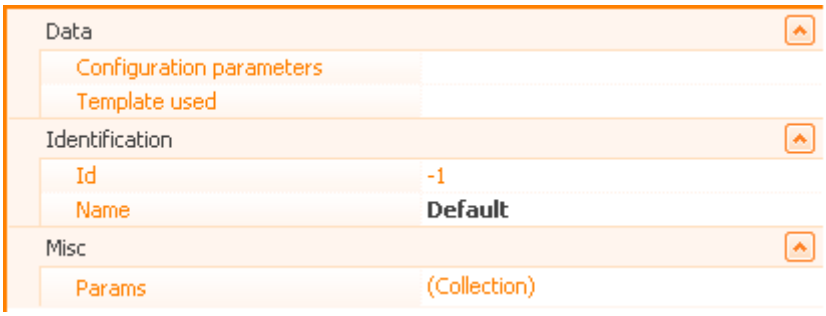


Figure 34 - Stage Manager: Default Configuration section

Example 2: Information for a well plate:

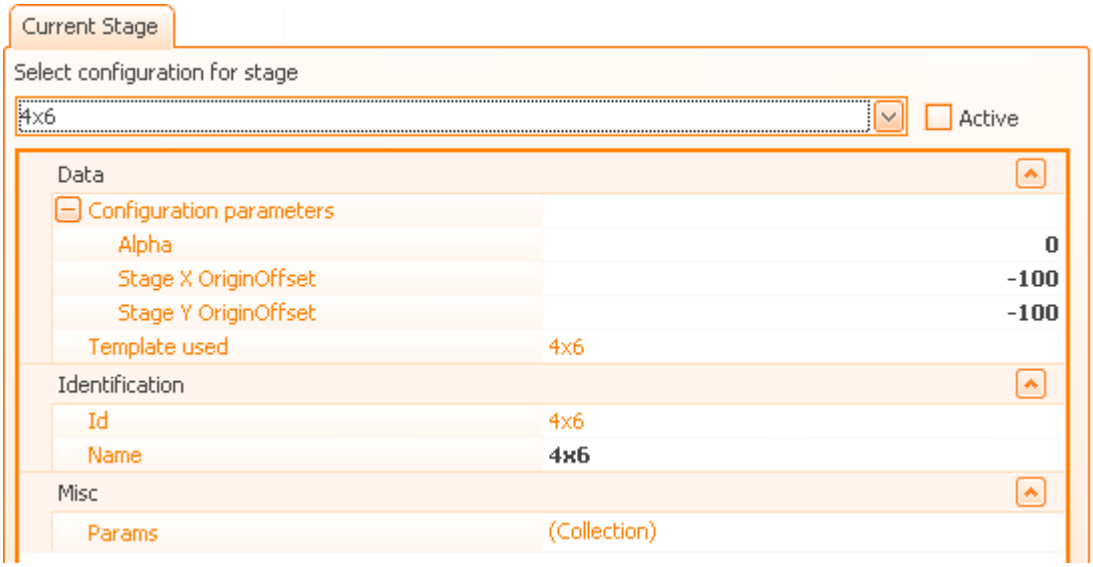


Figure 35 - Stage Manager: Well plate section

After you have selected the desired configuration you must click on the **Active** checkbox near the dropdown list.

Once you're done, you must press **Save and Exit**. Any existing experiment will be saved and closed. The selected configuration will apply only to newly created experiments.

Creating new configurations

In the drop down list with the available configuration, click on the entry **New...**.

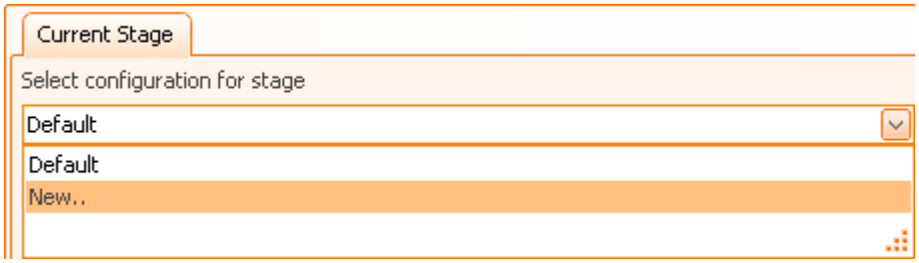


Figure 36 - Creating new configurations combo box

At this point, the **Create template** dialog will open. It contains two options:

1. Create template based on an existing template

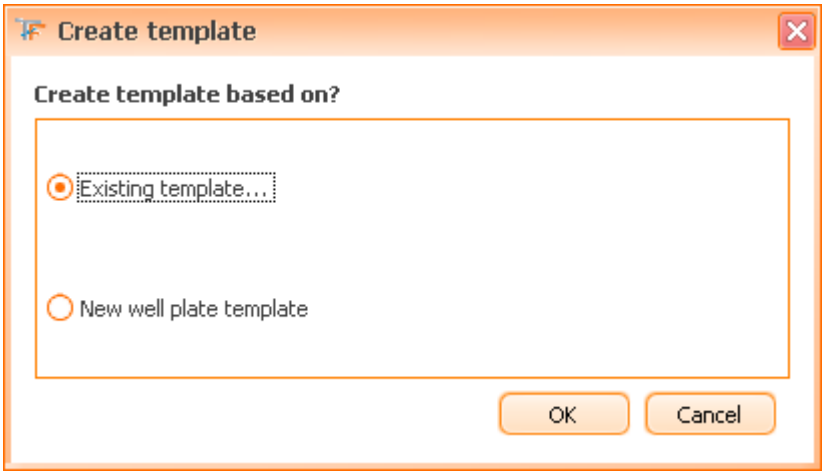


Figure 37 - Create well plate template dialog

A new window will appear. Note that information about the selected template is displayed below in the drop down box (our example shows a well plate insert).

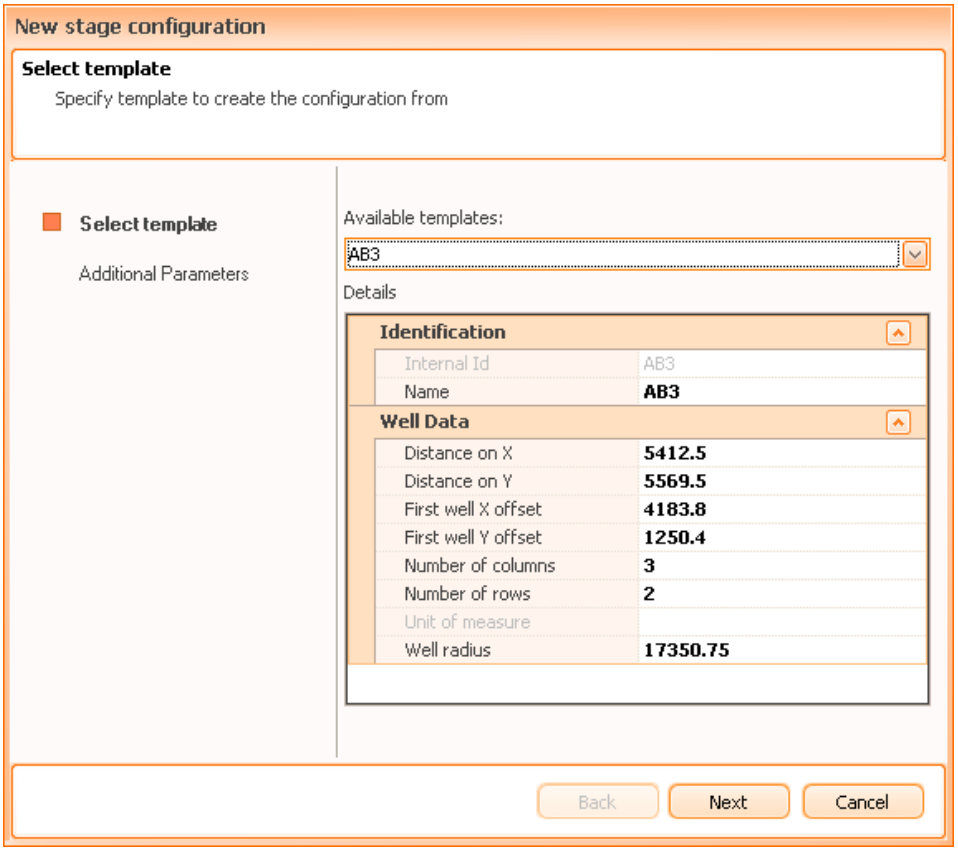


Figure 38 - New stage configuration: Select template dialog

At this point, you must choose one **template** from the available templates and click **Next**.

You must now specify the distance between the 0,0 point of the stage and the leftmost edge of the well plate, respectively topmost edge point of the well plate (actually the **Stage X Origin Offset** and **Stage Y Origin Offset** parameters, that you can see in the image below).

The **Alpha** parameter represents the skew angle of the well plate insert related to the stage (usually, this parameter is 0).

Performing this configuration for the well plates will make sure that the default regions created by **TissueFAXS** will match the actual well plates on the stage.

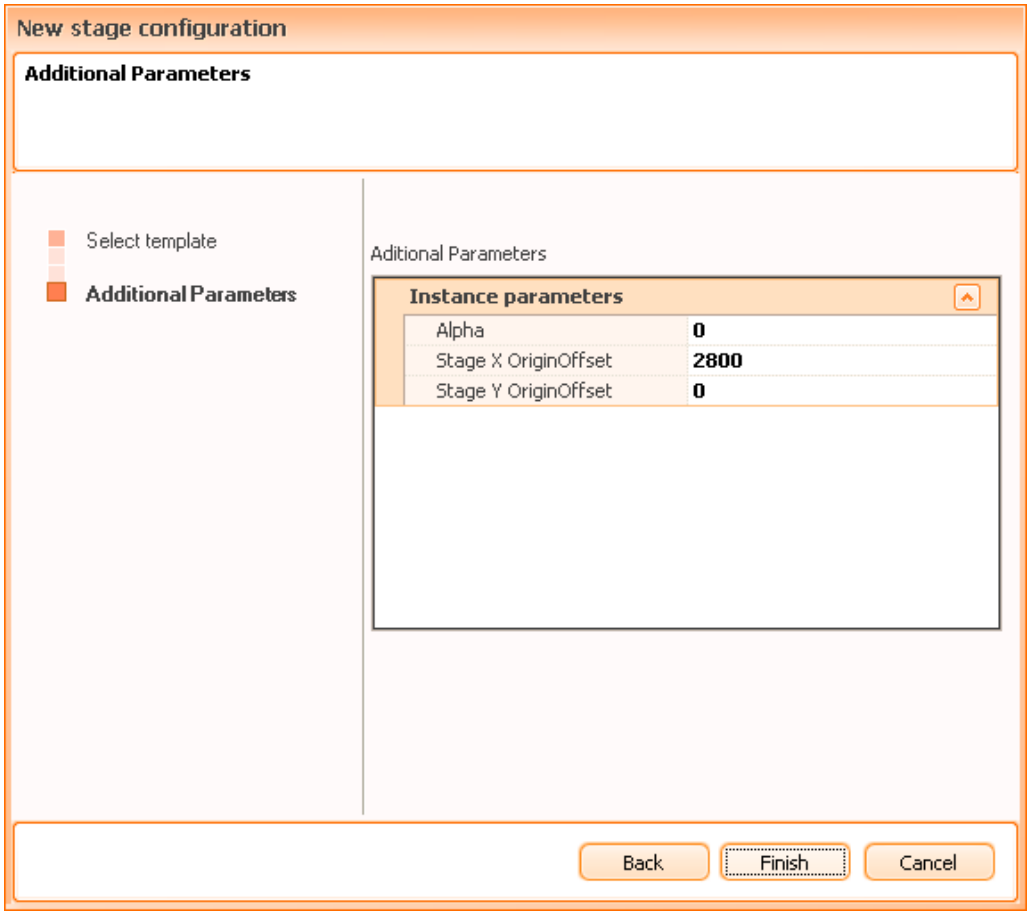


Figure 39 - New stage configuration: Additional Parameters dialog

Note

- In case of **slide inserts**, the distance is computed between the 0,0 point of the stage and the first slide on the insert.

Once you're done, you can select the newly created configuration using the previously described steps.

2. Create new well plate template

By choosing **New well plate template** (New well plate template) from the **Create template** dialog, you will be guided by a wizard to setup **TissueFAXS** for using a well plate insert.

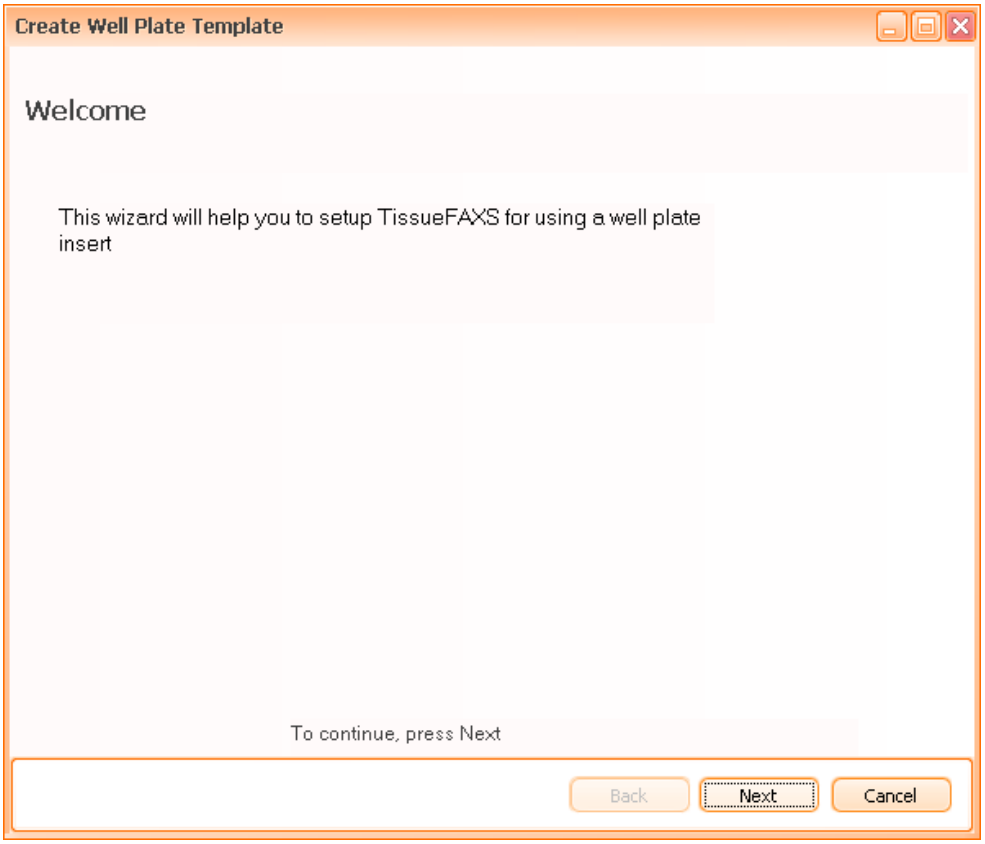


Figure 40 - Create well plate template: Wizard

Step 1: here you must choose the **name** and the **type** of the template to be created. For the well plate type, some options are already available by default, but you can also create a new type by choosing **Custom** option from the dropdown menu.

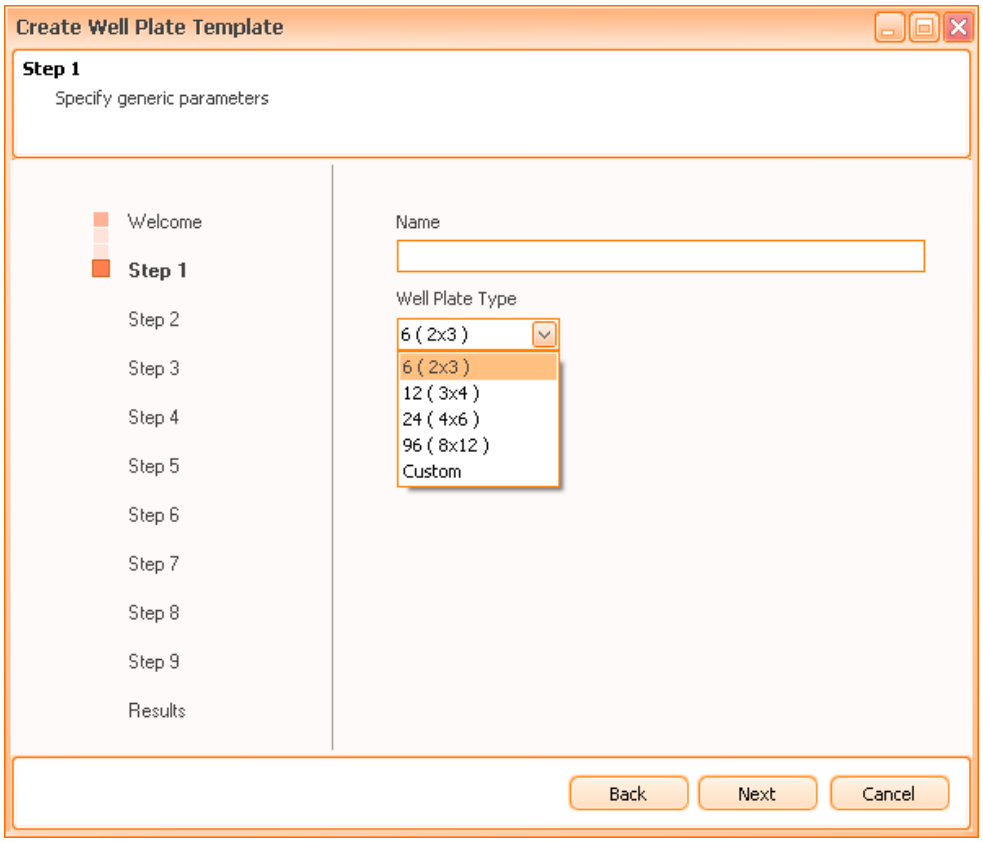


Figure 41 - Create well plate template: Wizard, Step 1

In the **next steps** of the wizard (2-9), simply follow the indications given for each step (see figure below).

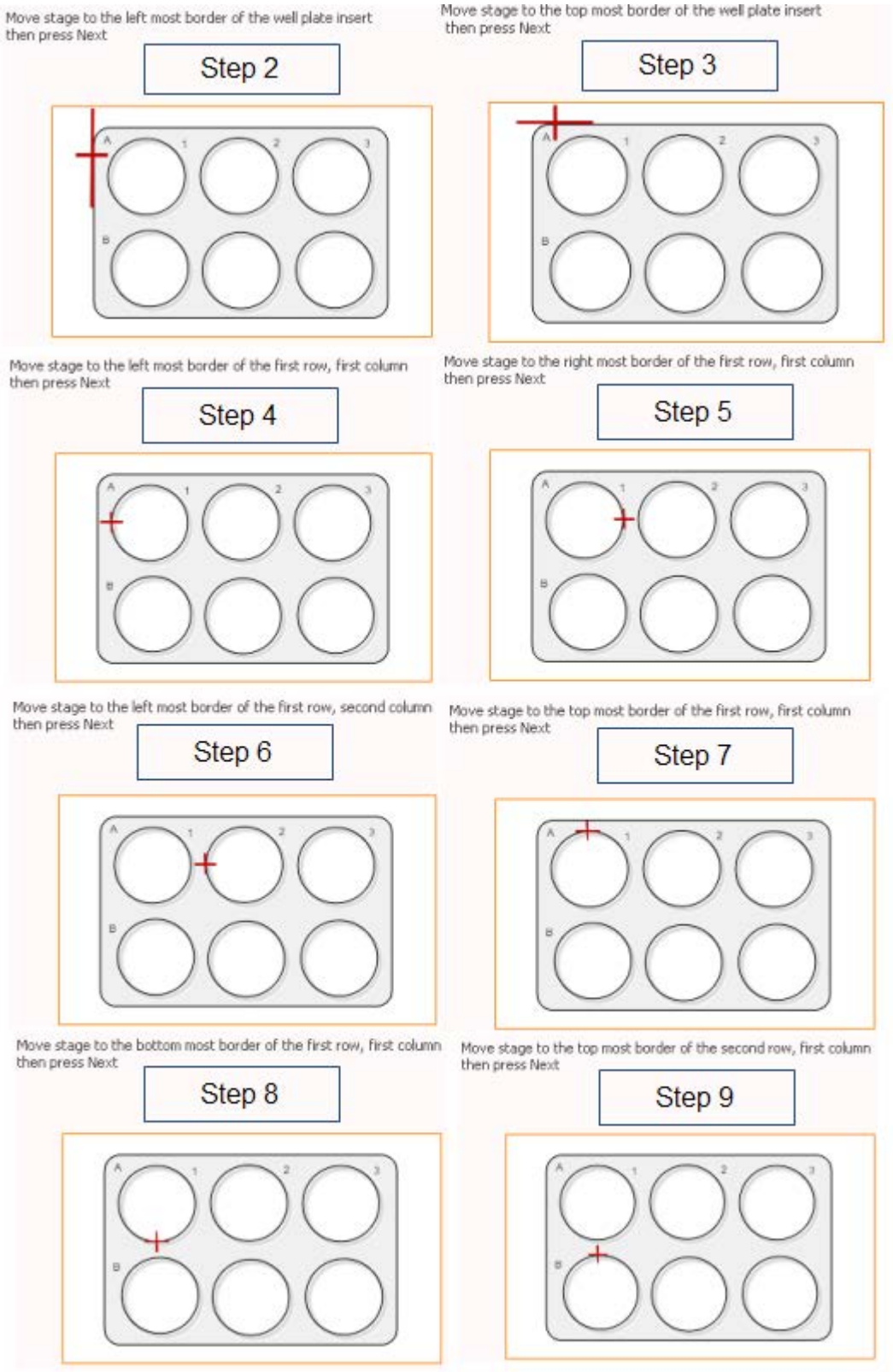


Figure 42 - Create well plate template: Wizard, Steps 2-9

Note

- Once you have created a well plate experiment and added the well plate regions, you can control their size and position in a similar manner as described in the TMA chapter

(please see **Chapter 6**).

- After creating the well plates templates, they will be available for further use in the **Drop down box** (Default).

4.4.4 Load Settings

The user can use this feature to load the settings from the previous version of the software.

CAUTION

- Use the **Load settings** feature ONLY if you are instructed by TissueGnostics personnel or authorized distributors.
- **Load settings** feature is only available if logged as technician or administrator.
- After the update is finished, the application will be automatically closed.

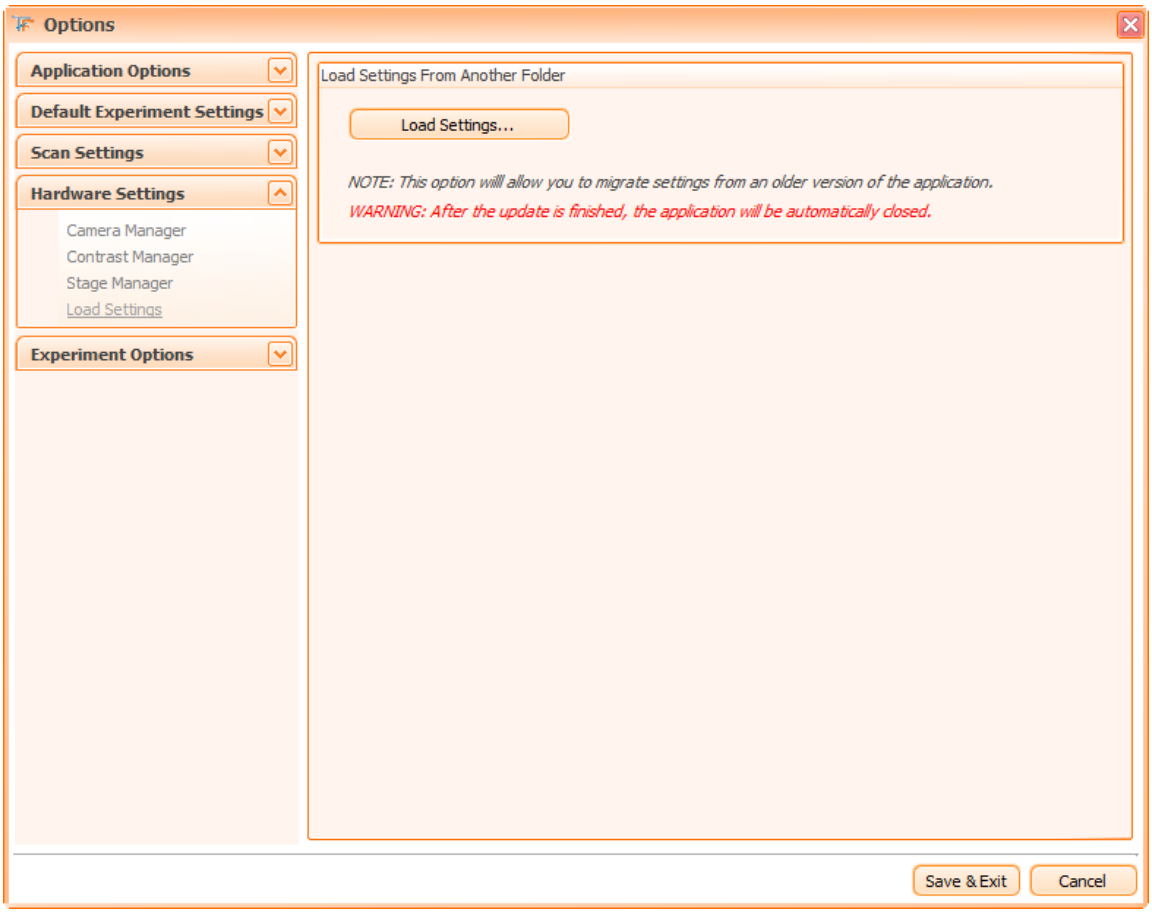


Figure 43 - Load Settings dialog

4.5. Experiment Options

4.5.1 Preview

This dialog resembles very much to the **Preview** dialog from the default options above. The difference is that changes in this dialog affect the actual experiment.

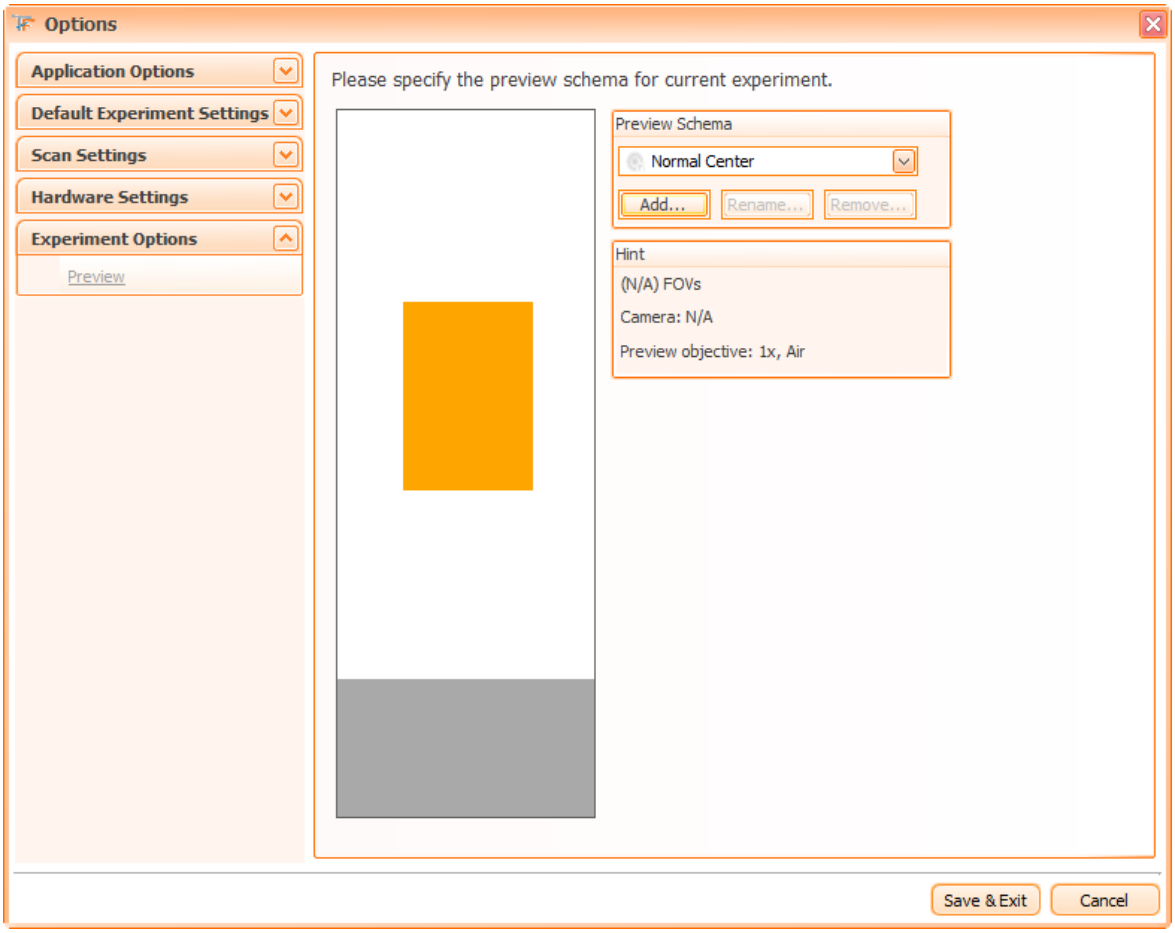


Figure 44 - Experiment Options: Preview dialog

4.6. Calibrate Field of View

The *camera calibration* is a basic, but essential operation when using **TissueFAXS**, as the quality of the acquired images directly depends on it. **We advice you paying maximum attention and perform the calibration with particular care.**

We recommend calibrating the size of the Field of View for each objective from **Tools → Calibrate Field of View…** menu.

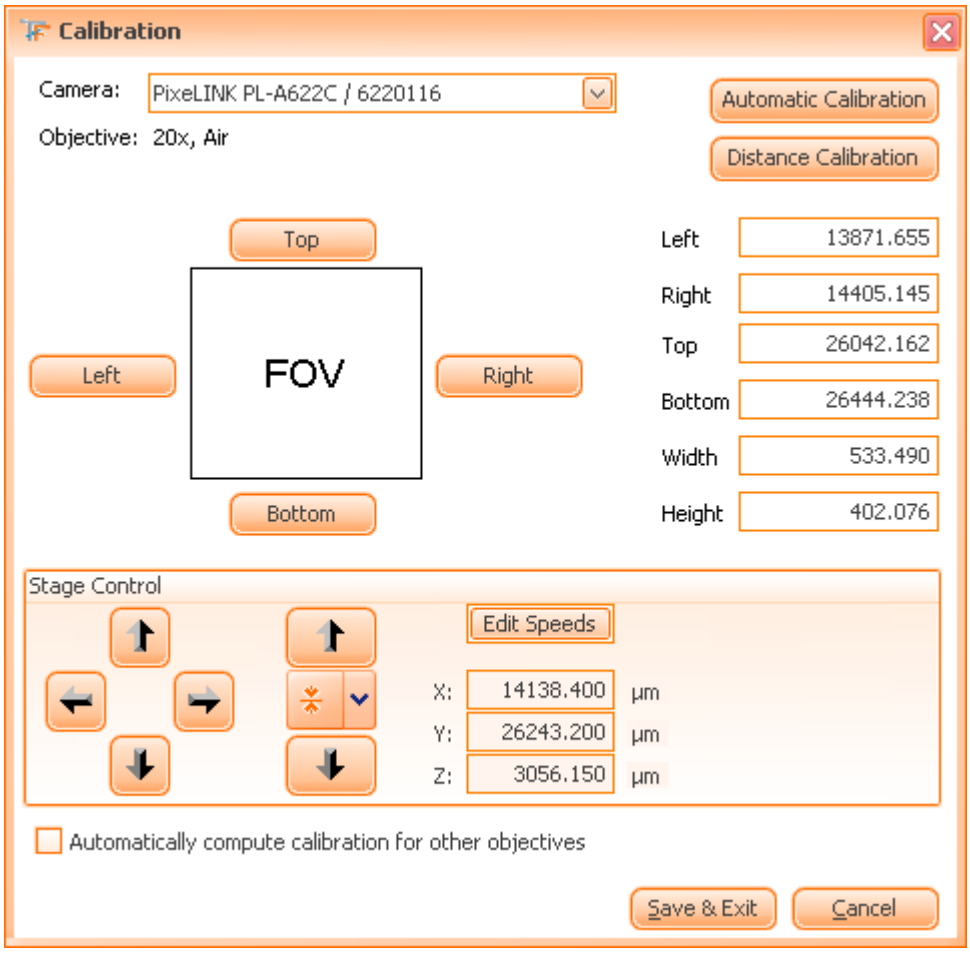


Figure 45 - Field of View Calibration control panel

This dialog allows you to compute the size of the Field of View for the current objective.

In order to accomplish this task, you have **three possibilities**:

- manual calibration;

- automatic calibration;

- distance calibration.

Note

- For all FOV calibration methods, it is recommended to use a slide containing a more prominent area, in order to track it easier.

- For optimal results, you may use a calibration slide (the calibration slide is mandatory for the *distance calibration* method).

4.6.1 Manual FOV Calibration

- Choose a *reference point* on the sample. For a good evaluation of the reference point, please make sure the whole object is in focus;
- Move the reference point in the upper-right corner by using the software or hardware joystick;

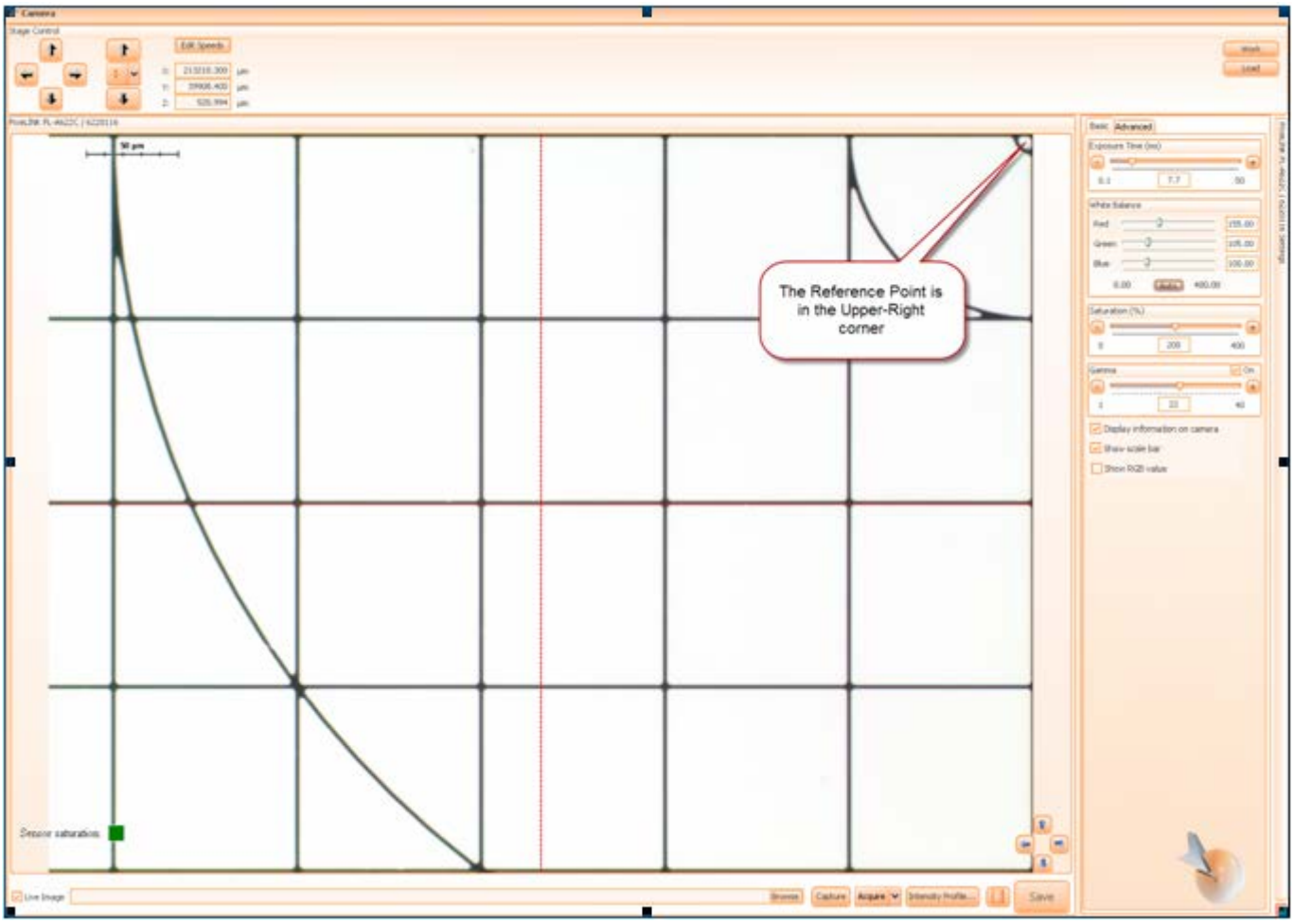


Figure 46 - Reference Point: Upper-Right Corner

- Press **Top**, then **Right**;

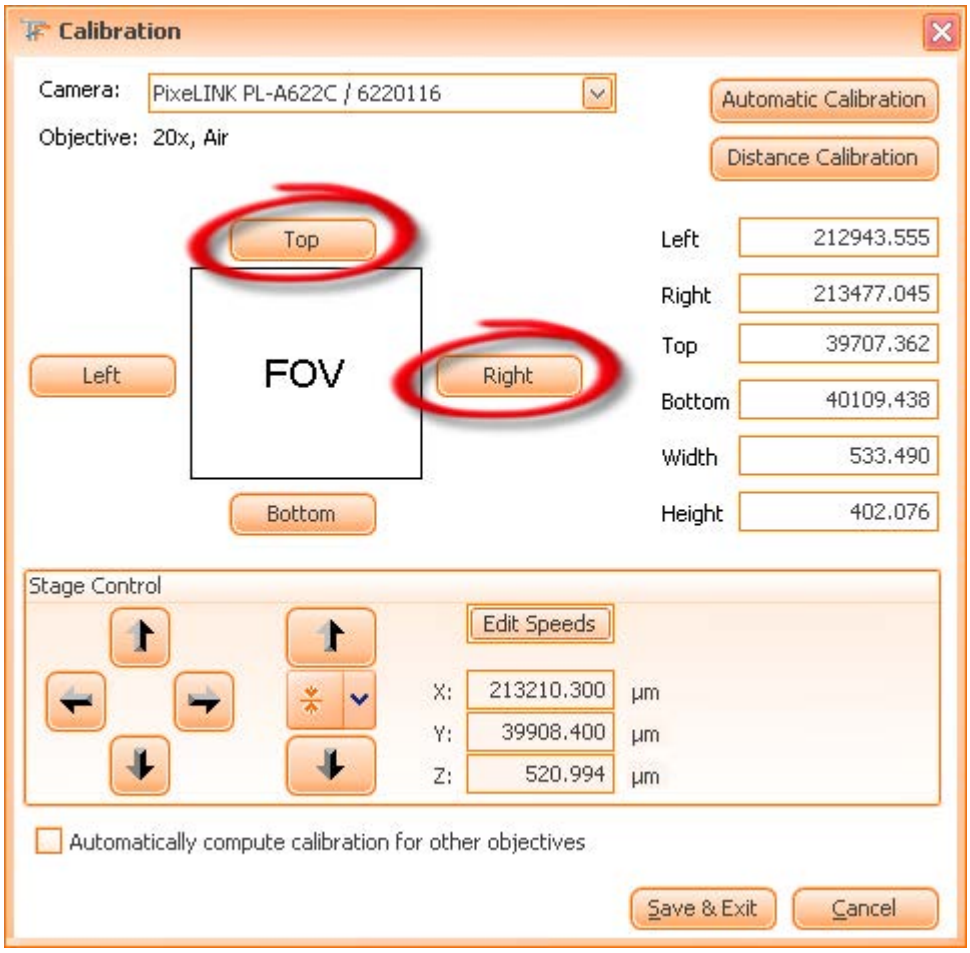


Figure 47 - Manual calibration (a)

- Using the joystick, move the reference point from the upper-right corner to the lower-left corner;

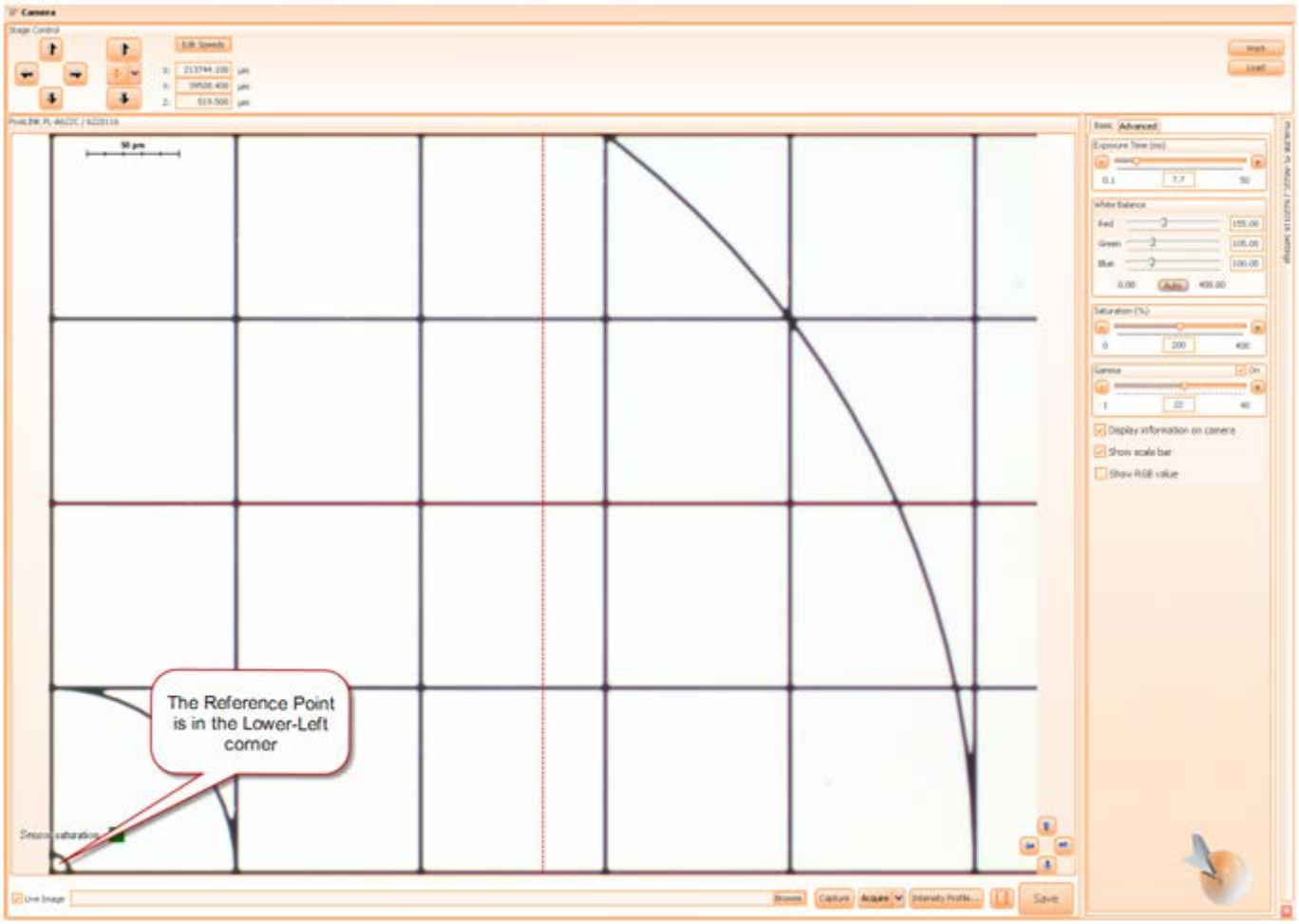


Figure 48 - Reference Point: Lower-Left Corner

- Press **Bottom**, then **Left**. At this point, the **Width** and the **Height** values will automatically be computed;
- To save the computed values, press **Save and Exit**.

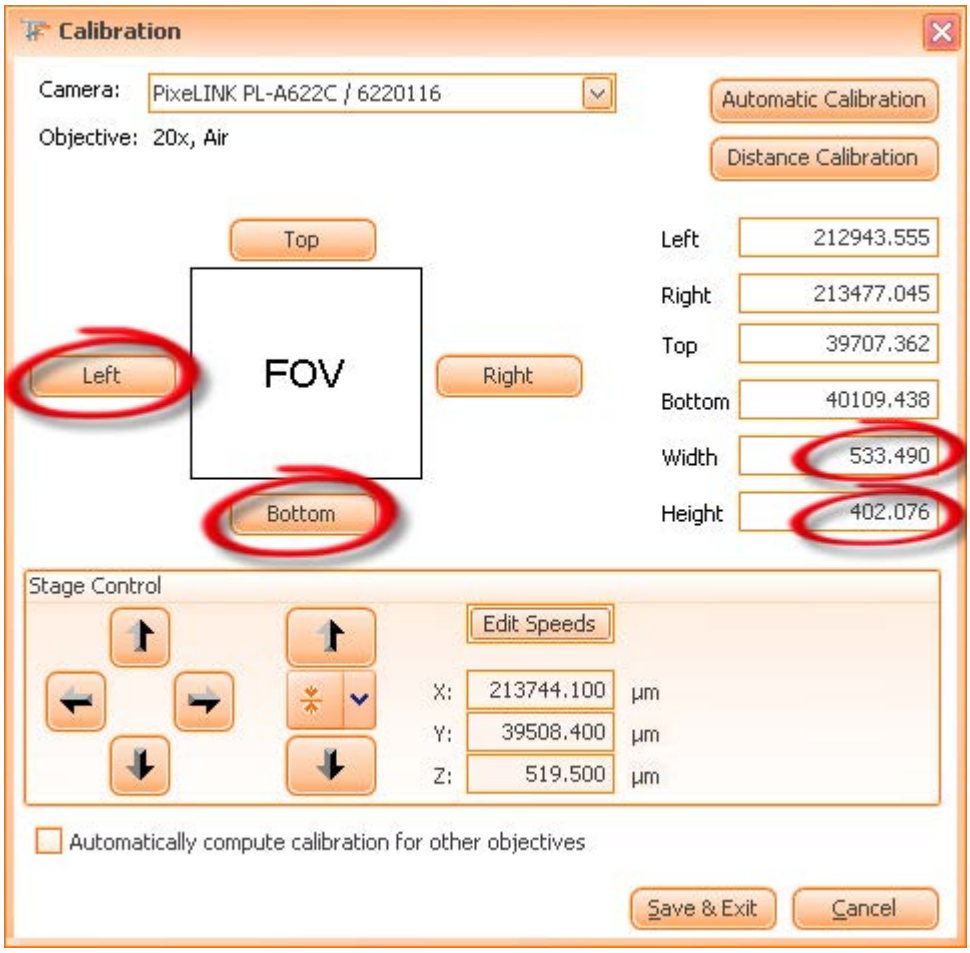


Figure 49 - Manual calibration (b)

TIP - If you already know the FOV size, you can introduce the **Width** and **Height** values by yourself in their corresponding fields.

4.6.2 Automatic FOV Calibration

- Choose a reference point on the sample. For a good evaluation of the reference point, please make sure the whole object is in focus.

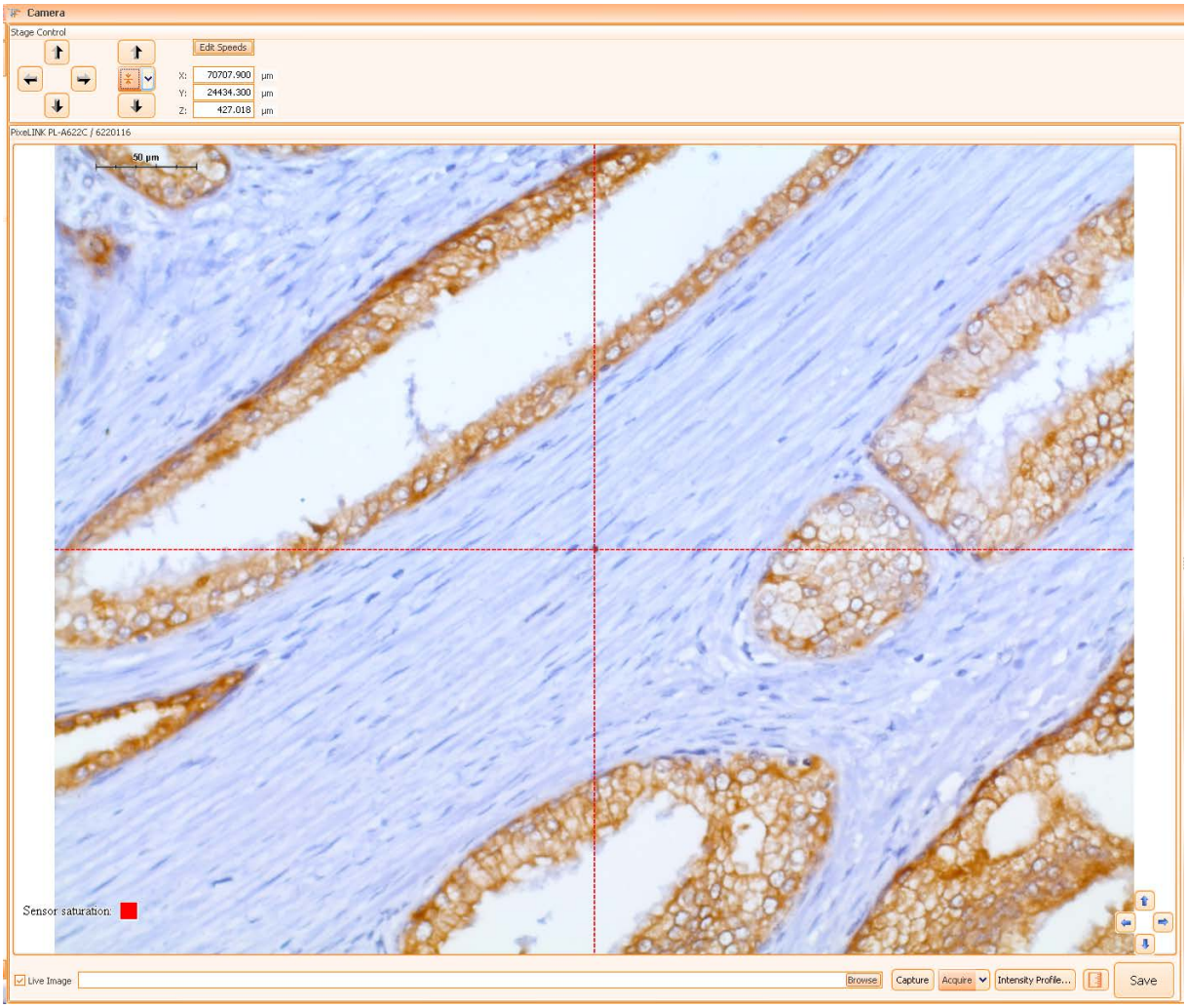


Figure 50 - Generic tissue sample. The point in the center is the reference object.

If using a calibration slide, place the center of the calibration slide on the live image like in the figure below.

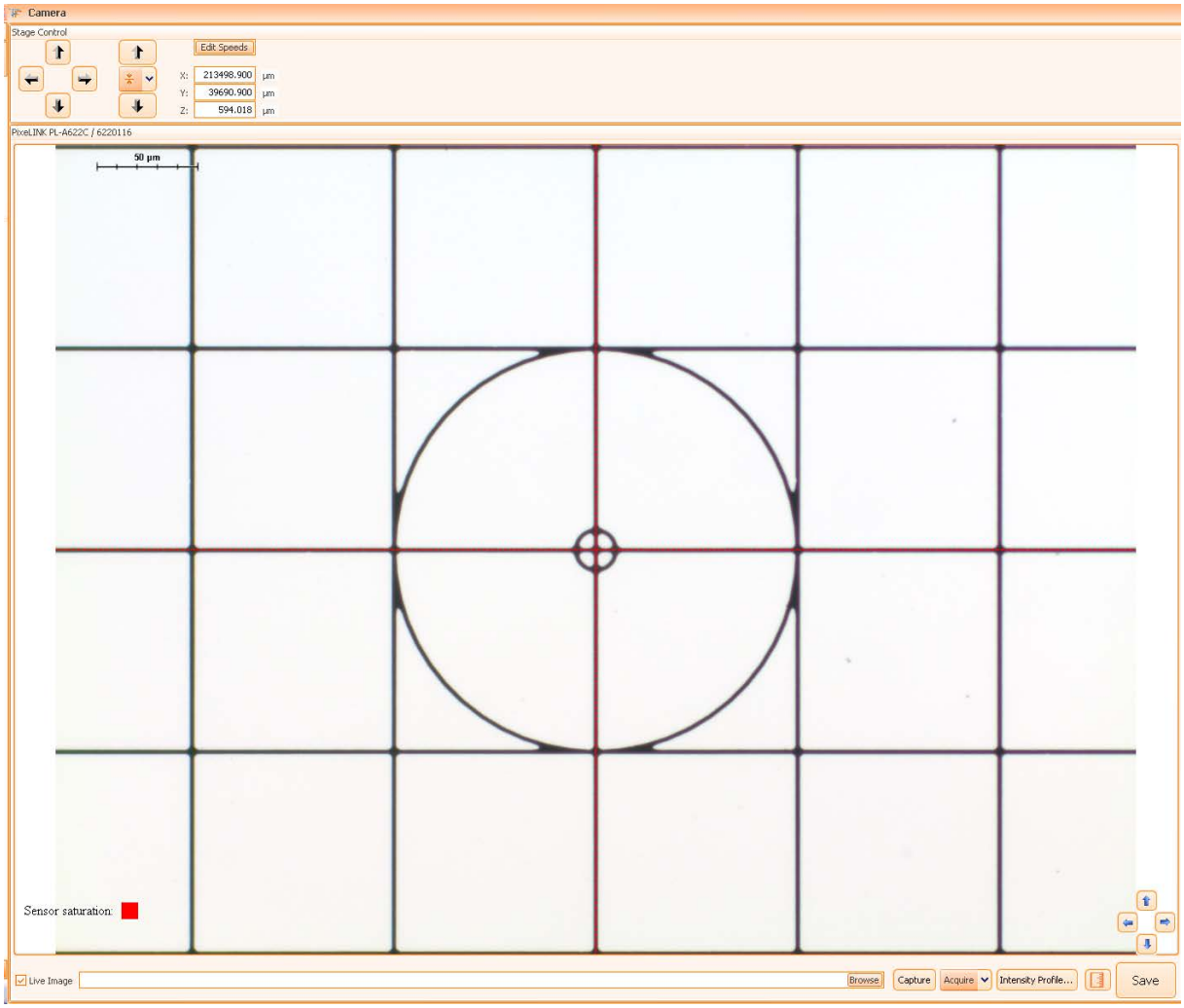


Figure 51 - Calibration slide centered

Note

- In order to bring up the red cross (marking the center of the calibration slide) on the live image, use the key combination ***Alt+C***.

CAUTION

- Do not use images with repeating patterns! (For instance, slides with fluorescence beads might contain many similar objects and auto calibration will fail or return wrong results.)
- Ensure the speed of the stage is low!

- Press **Automatic Calibration** button. The stage will be moved and the software will determine the following:
  - The correct flip settings. You will be prompted only if the current flip settings are wrong.

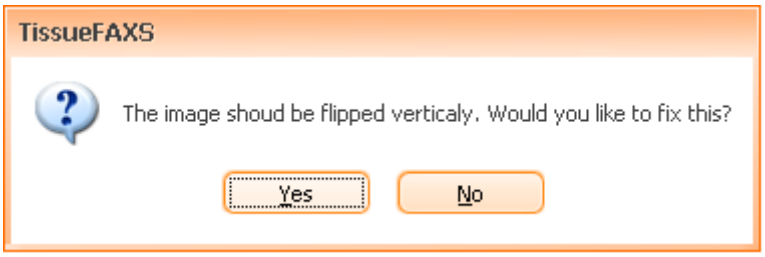


Figure 52 - Message warning that the current flip settings on camera are wrong

- The angle that is formed when the camera is misaligned. You will need to manually rotate the camera and rerun the calibration to recheck this.
- The dimensions of the FOV.

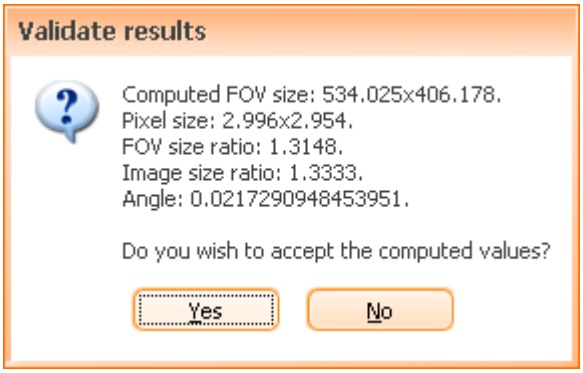
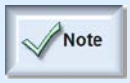


Figure 53 - Output of the Automatic FOV calibration

- For best results, set the speed of the stage at a minimum value. When using other slides than calibration slides, you can run this procedure on different areas to ensure the correct value is computed.



- If the image is flipped, the algorithm will detect this and will set the image to the right position.

- To save the computed values, press **Save and Exit**.

4.6.3 Distance Calibration

- Insert the calibration slide in an empty insert position;
- Choose the objective for which you want to perform the calibration;
- Enable the live image, focus and center the calibration slide on the live image;
- Access the calibration function: from **Tools** (Tools) menu, choose **Calibrate Field of View…** option:

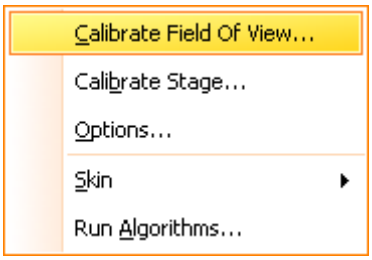


Figure 54 - Tools menu

- The **Field of View Calibration** control panel will appear:

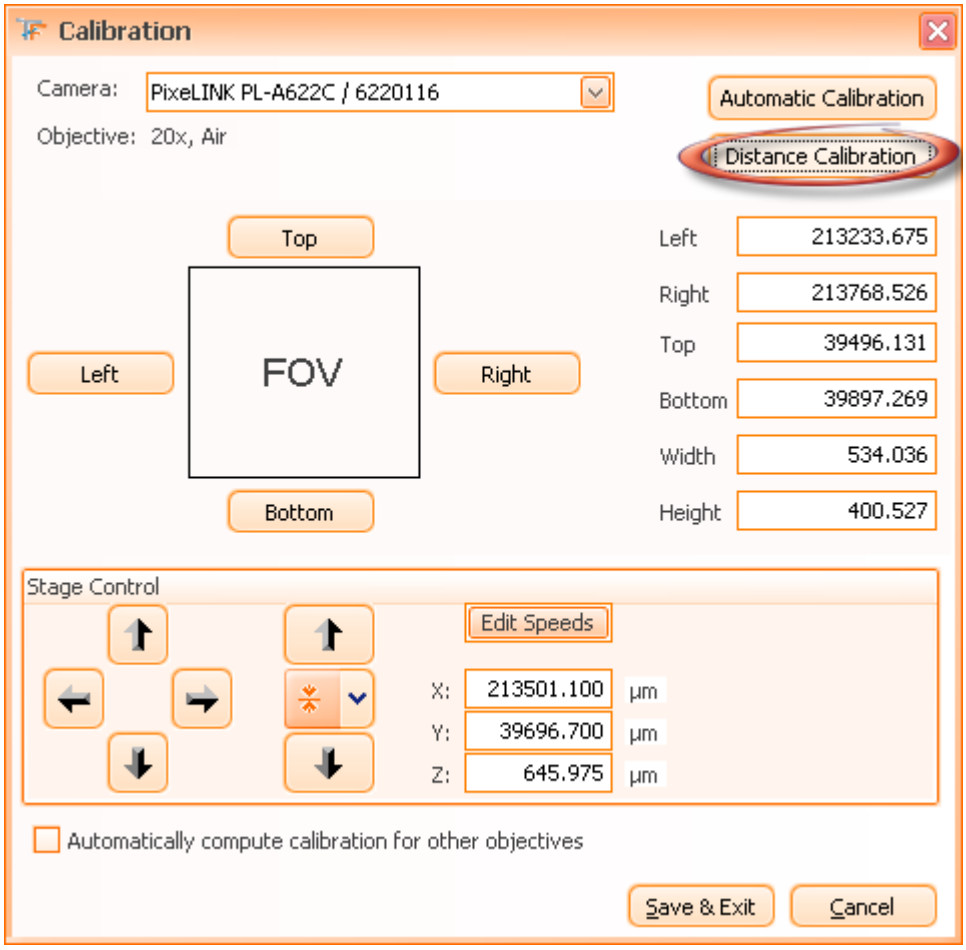


Figure 55 - Field of View Calibration control panel

- Now press **Distance Calibration** button (

) and the **Distance Calibration** panel will appear.

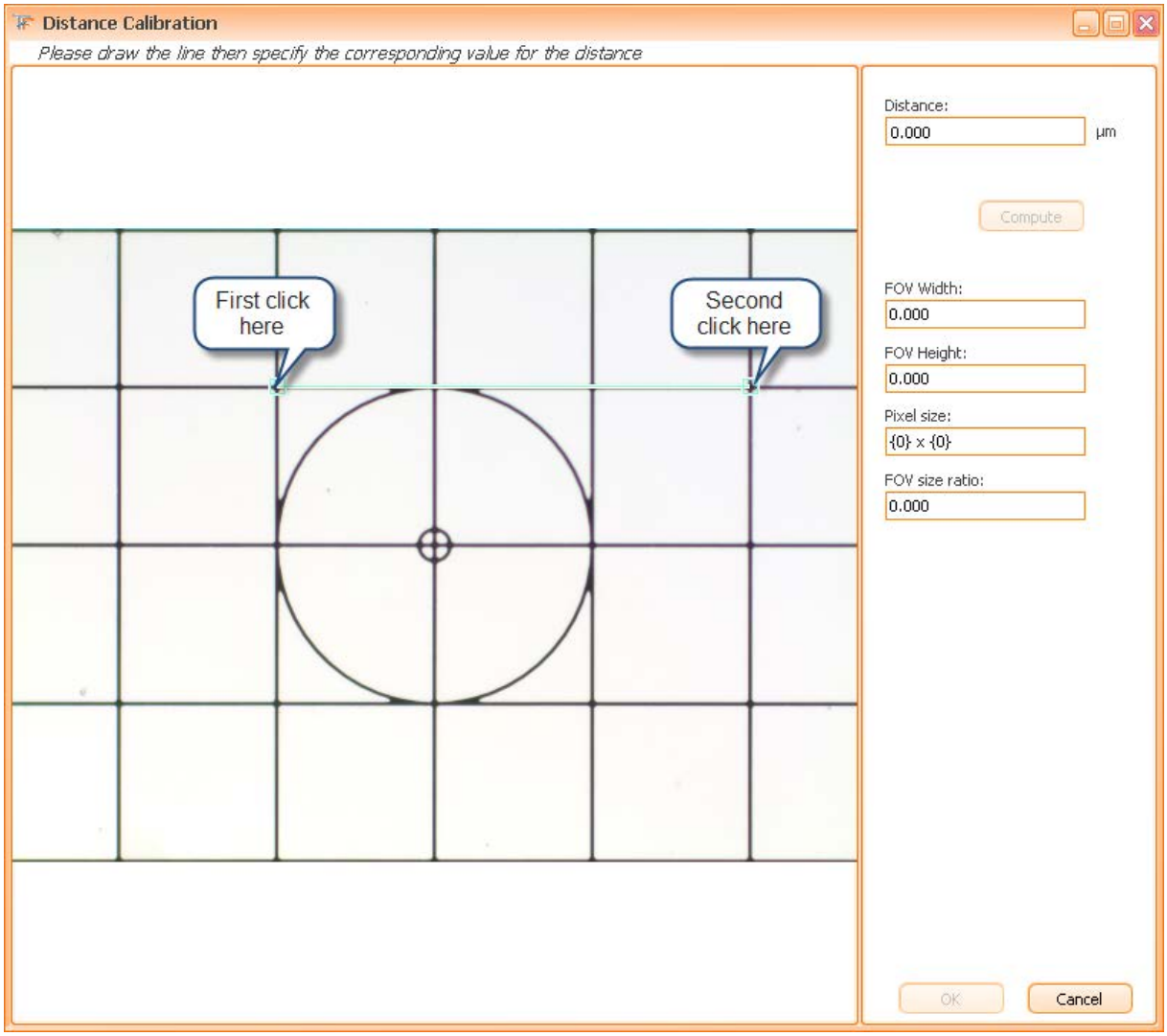


Figure 56 - Distance calibration panel (a)

- Draw a line between two points for which you know the distance. Click in a desired point to start drawing the line, then click again to determine the end of the line. In our example, the squares on the calibration slides are 100μmX100μm. Use a distance as big as possible in order to improve results;
- Specify the distance between the two selected points (the value must be in μm);
- Press **Compute** button: FOV Width, FOV Height, Pixel size and FOV size ratio will automatically be computed;
- Press **OK** button in order to save the obtained values.

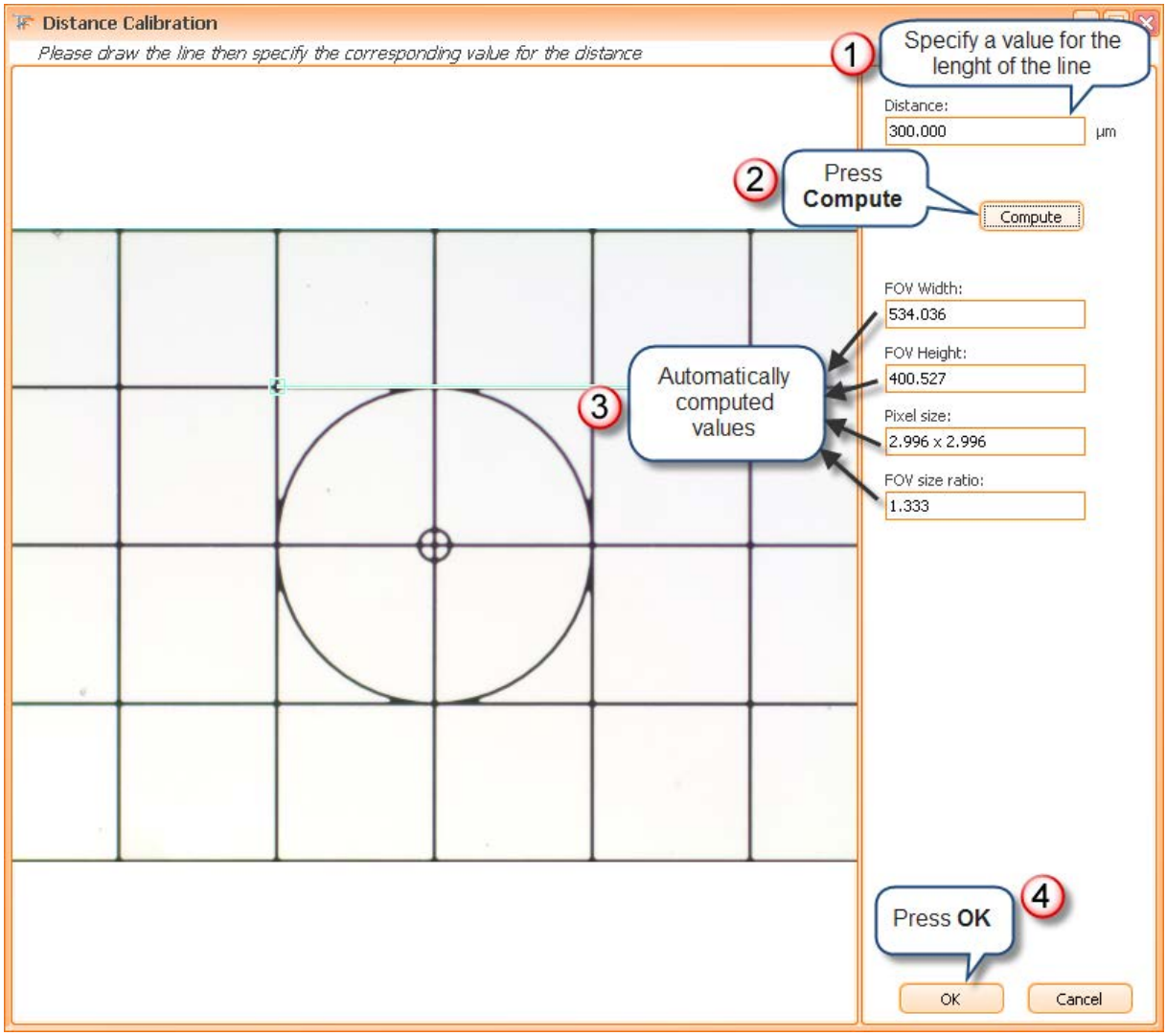


Figure 57 - Distance calibration panel (b)

About calibration methods:

- Only the FOV values for the current objective have been calculated / recalculated this far. In order to compute the FOV sizes for the rest of the objectives, repeat the steps for each objective. For a quick setup, but less precise, you can select the objective with the medium magnification between your objectives, compute its FOV size and, before pressing **Save and Exit** (Save & Exit), select **Automatically Compute calibration for other objectives**.
- At any time, you can press **Cancel** if you don't want to save the changes.

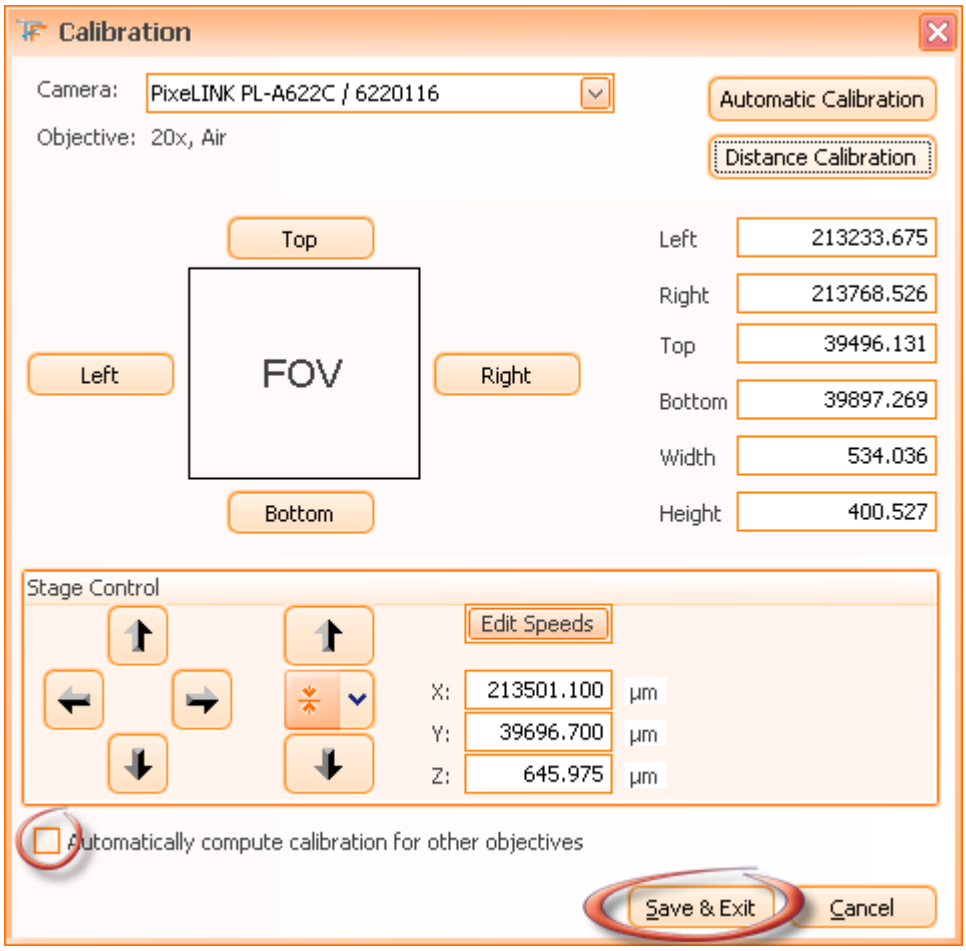


Figure 58 - Field of View Calibration control panel: final operations

4.7. Users

In order to use **TissueFAXS**, you must be authenticated, by owning a username and a password.

4.7.1 View Users

To manage the users, you must be logged in as Administrator. In **TissueFAXS**, there is only one Administrator and a set of users, who may be managed in the application when the Administrator is logged in.

From the main menu, choose **Admin**, then **Users**, and a panel will appear:

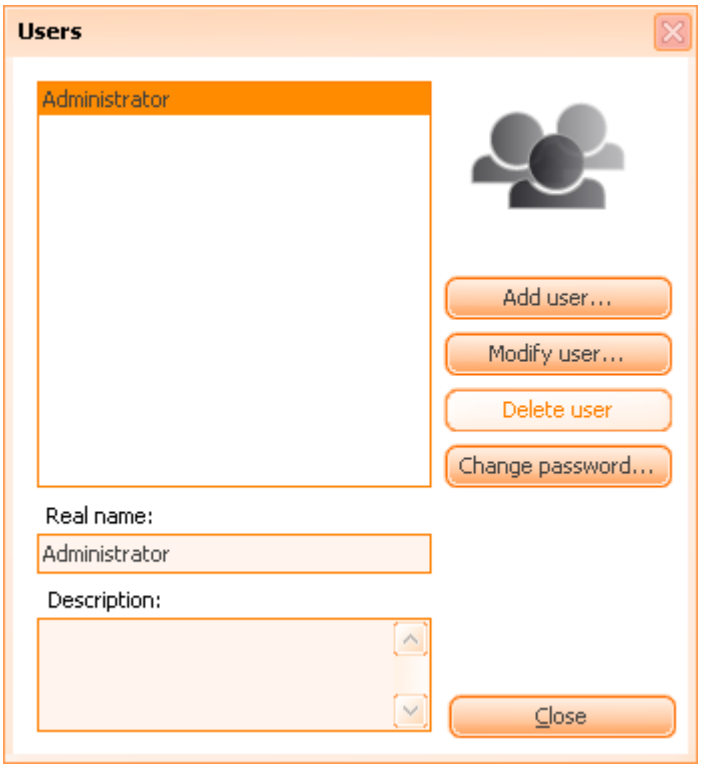


Figure 59 - Users dialog

4.7.2 Adding Users

Click on the **Add user** button and you will get the following panel:

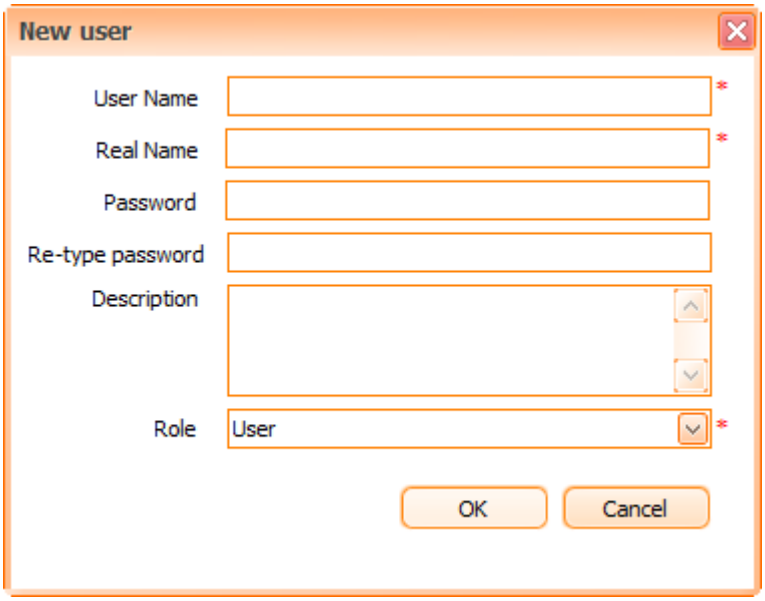


Figure 60 - Adding Users dialog

Here, you can fill necessary information concerning the new user, as the **User Name**, the **Real Name**, the **Password**, **Description** and **role**.

Note - All fields, excepting **Description**, are mandatory.

4.7.3 Modifying Users

By pressing **Modify user** button, you can use the panel that appears to update the real name, the description and the role of the user.

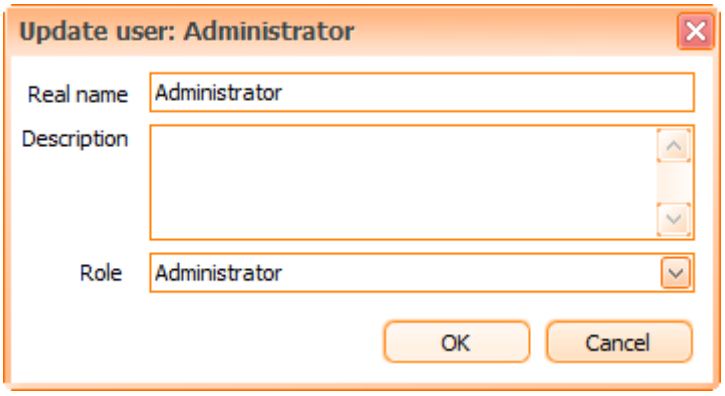


Figure 61 - Modify User dialog

4.7.4 Delete Users

To delete a user, select it in the **User list** dialog, then press **Delete user button**. You will receive a final warning before the effective deletion; it looks like the image below:

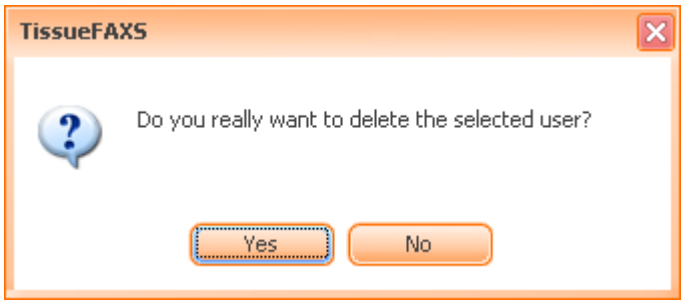


Figure 62 - Delete User message box

4.7.5 Change Password (Administrator Mode)

When you are logged in as Administrator, you can change other users' passwords by using the dialog shown in the image below. To change the password, the application will first ask you to type the **old password**, then the **new password** and its confirmation.

Figure 63 - Changing other user's password dialog

4.7.6 Role Based Administration

In order to prevent certain untrained users to access the advanced controls of the application, a role based policy exists in **TissueFAXS**.

Role based administration implies access restriction to non administrators for advanced controls of the application.

The roles can be assigned in two ways:

- When creating a new user, choose one of the three available options from the **Role** dropdown box:

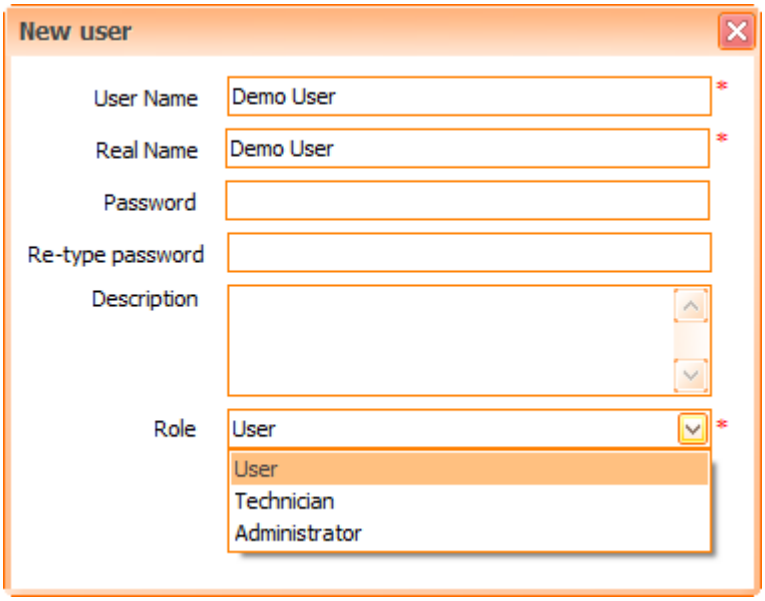


Figure 64 - New User: assigning role

When modifying a user, choose one of the three available options from the **Role** dropdown box:

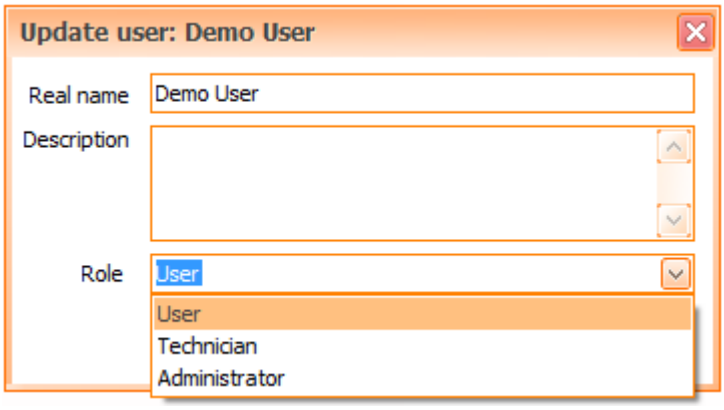


Figure 65 - Modify User: assigning role

The following user roles are available:

1. User

- This role cannot modify wide system settings like FOV calibration or scan strategies.

2. Technician

- This role can modify wide system settings like FOV calibration.
- Inherits all the rights of the User role.

3. Administrator

- This role can modify the list of users and their roles.
- Inherits all the rights of the Technician role.

List of settings and the minimum access level:

If using the **Role Based Administration** feature, some settings will be available only for Administrator or Technician. Please see table below for more details:

Operation	Minimum Access Level	Behavior for users without access
Run Algorithms	Technician	Hidden
Calibrate Field Of View	Technician	Hidden
Test Microscope	Technician	Hidden
Test Stage Precision	Technician	Hidden
Administrate Users	Administrator	Hidden
Options → Default Experiment Settings → Storage → Raw Data	Technician	Disabled
Options → Scan Settings → Scan Strategy	Technician	Disabled
Options → Scan Settings → Speed	Technician	Disabled
Options → Application Options → Actions → Move To a Safe distance	Technician	Disabled
Options → Application Options → Actions → Calibration	Technician	Disabled
Options → Application Options → Support → Connection Details	Technician	Disabled
Options → Application Options → Support → Test Connection	Technician	Disabled
Camera → Advanced	Technician	Hidden
Options → Application Options → Licensing	Technician	Disabled

5. Using TissueFAXS

5.1. New Experiment Wizard

In order to acquire images in **TissueFAXS**, it is essential to create a new experiment or open an existing one. All images, settings and data are saved and embedded in your experiment files and folder.

The only thing you can do if you don't have a current experiment opened is to preview slides or acquire single images from the camera window.

Opening an existing experiment

To browse for an existing experiment press **Open** button ( ) from the toolbar or press **Open…** from the **File** menu ( ).

To access the recently used experiments press **Open** button arrow ( ) from the toolbar or press **Open…** arrow from the **File** menu ( ):

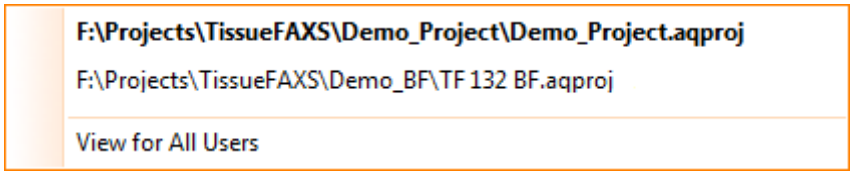


Figure 66 - Recent Experiments list

From this list, choose the desired experiment and left click on its name to open it.

Currently opened experiment name appears bold on the list.

If you choose **View for All Users** option, **Recent Experiments** dialog will open. Here you can see the previously opened experiments by other users (see **Chapter 4.7.6**) and you can open any experiment by double clicking on it.

You can delete items from the **Recent Experiments** dialog by selecting desired item(s) ad pressing **Delete** button from your keyboard. You can also use the contextual menu that appears when right-clicking on any item in the list.

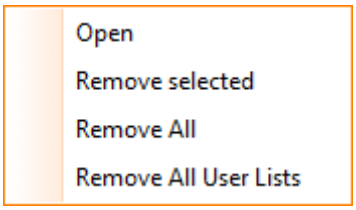


Figure 67 - Recent Experiments: contextual menu

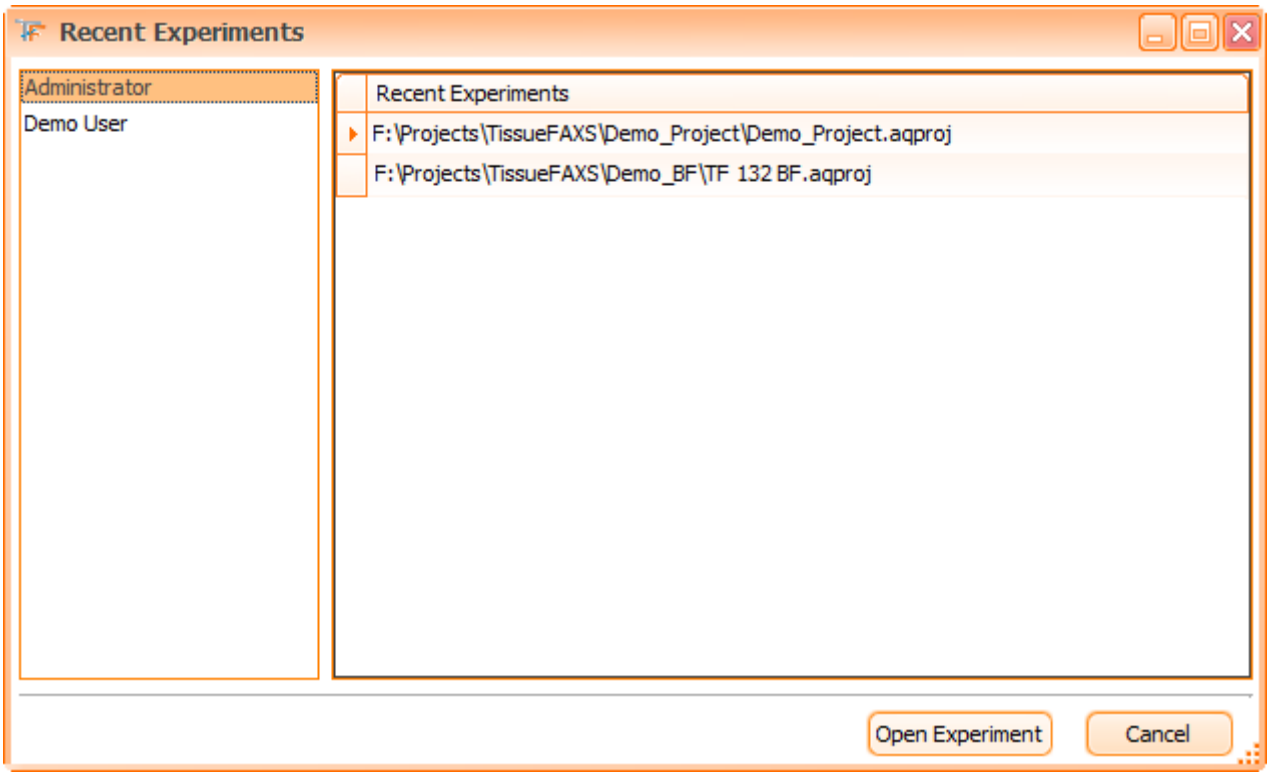


Figure 68 - Recent Experiments dialog

5.1.1 Experiment Creation

It's easy to create a new experiment, as **TissueFAXS** will guide you through a 5-step user-friendly wizard.

- To begin creating a new experiment follow the steps below:

Go to **File** → **New…** menu:

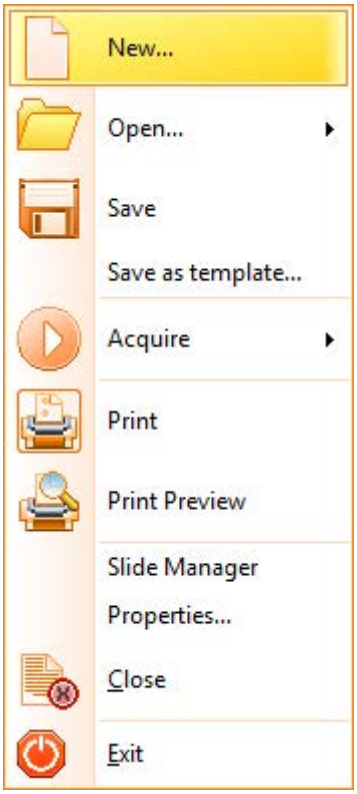


Figure 69 - New Experiment menu

Or go directly in the toolbar:

Figure 70 - New Experiment (toolbar)

At this point, you have **two possibilities** to create a new experiment:

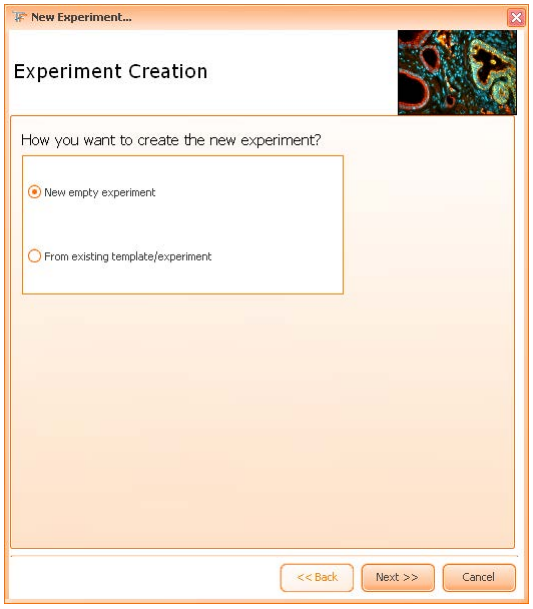


Figure 71 - Experiment Creation

- If you choose to create an empty experiment, follow these steps: **Experiment Identification**, **Experiment Folder**, **Experiment Settings** and **Finish: Experiment Ready**.
- If you instead choose to start from a template or an existing experiment, follow these steps: **Browse for template/experiment**, **Experiment Identification**, **Experiment Folder**, and **Finish: Experiment Ready**.

5.1.2 Browse for template/experiment

In this page of the wizard, you must browse for a template or an existing experiment. This will conveniently copy most of the useful data, including the settings for preview and acquisition channels, to the new experiment.

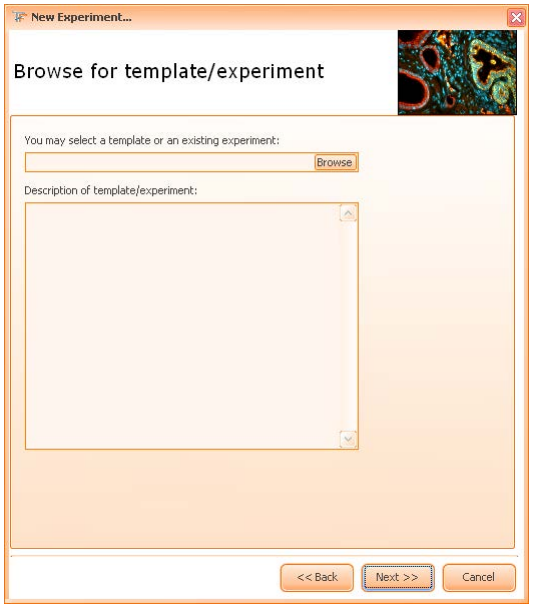


Figure 72 - Browse for template/experiment

After choosing the template, a short description is displayed.

You can find below the **data** from the template/existing experiment used for the new experiment:

- Experiment type;
- Preview objective;
- Preview channels;
- Acquisition objective;
- Acquisition channels;
- Camera;
- Preview schema;
- Label position.

An experiment can be **saved as template** by accessing the main menu **File** → **Save as Template…**.

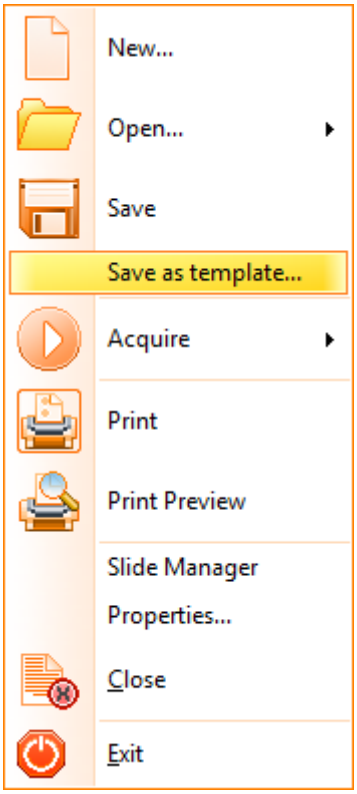


Figure 73 - Save as template menu

For saving the template, you must select a path for the file in the dialog that appears. The template file is saved as **.aqtpl**.

5.1.3 Experiment Identification

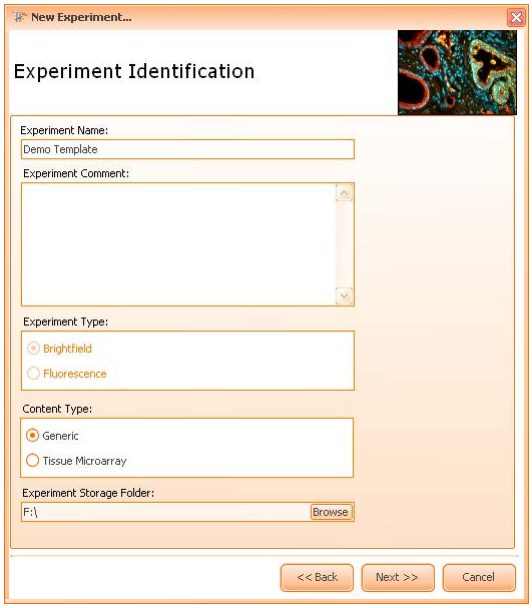
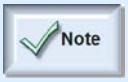


Figure 74 - Experiment Identification

In this page of the wizard the user will establish data that will define the characteristics of the experiment:

- **Experiment Name** (mandatory): the corresponding **TissueFAXS** experiment file will have this name and it will be stored in a folder having by default the same name as the experiment (it can be modified later).
- **Experiment Comment** (optional): you can add a short description of the experiment.
- **Experiment Type** (mandatory): you can choose what type of experiment you will create - Brightfield Experiment or Fluorescence Experiment. If you have created a new experiment from a template/existing experiment, the experiment type will already be selected and cannot be modified.
- **Content Type** (mandatory): you can choose a **Generic Experiment** or a **Tissue Microarray (TMA) Experiment**.
- **Experiment Storage Folder** (mandatory): to change the experiment storage folder just click on the label and then browse for the desired folder.



- Please ensure that you have enough rights on the selected folder.
- Please ensure that you have enough free space on the drive where the folder is located. The free space required depends on the file image format but at least 10 GB is recommended.

5.1.4 Experiment Settings

In this step, you can set some **properties** of your experiment:

1. Objective to be used for preview;
2. Objective to be used for acquisition. The default acquisition objective is set to 20x;
3. Reflectors used for acquisition;
   - In **Brightfield Experiments**: no selection can be made here. The channel **Transmission** is selected by default (it is specific for the Brightfield Experiments);
   - In **Fluorescence Experiments**: you can select the desired reflectors here. You can also modify the reflectors later from the **Acquisition Settings** tab;
4. Preview area from **Preview Settings** button;
5. Camera used for acquisition. A default camera is selected for each type of experiment. The default camera for brightfield experiment is the PixeLINK camera and for fluorescence experiment is the PCO PixelFly camera.
   - Settings for the preview operations can be set by clicking on the **Preview Settings** button (

). You can choose a preview schema from the list and select the position of the label from the slide (up or down). This slide label setting will remain the same for the entire experiment. It can only be changed later for single slides.

In addition, you can also select a profile with predefined settings from the dialog.

All settings made here can be changed later if necessary, only the experiment type cannot be changed.

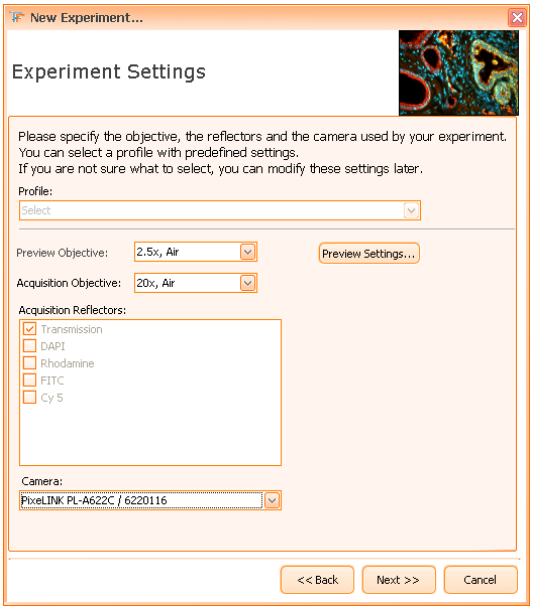


Figure 75 - Experiment Settings for Brightfield Experiments

You may notice that for Brightfield Experiments, the **Transmission** reflector is selected by default. A check box for **Transmission** indicates that no reflectors are present and the microscope is set to regular transmitted light.

The other reflectors are present in the list but they are disabled for **Brightfield Experiments**.

In **Fluorescence Experiments**, the panel looks like the figure below:

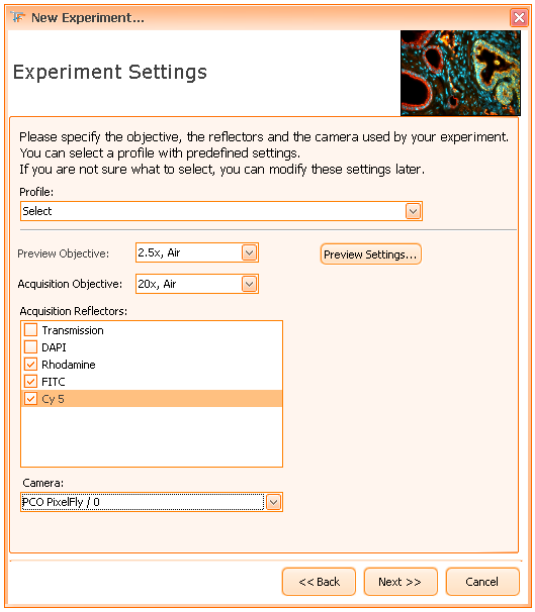


Figure 76 - Experiment Settings for Fluorescence Experiments

In this case, there is a list of reflectors that may be individually selected. For both types of experiments, you can adjust the preview area size by clicking on the Preview Settings... button. The preview area (or yellow box) can be manually adjusted with the mouse or you may select a pre-defined schema from the drop-down menu.

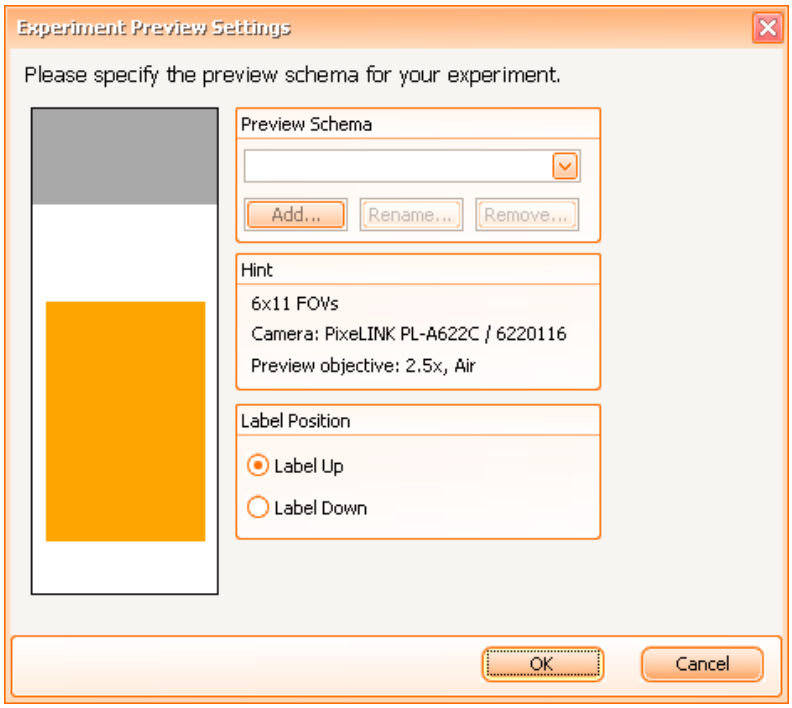


Figure 77 - Preview Area Settings panel

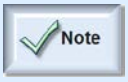
You can give a name to your **Preview Schema** and save it for future projects. This is useful when you have multiple slides which frequently have the region of interest (tissue) in the same specific location. Defining your preview schema over your region of interest will save time; otherwise the microscope will scan the entire slide in order to preview it.

The **Hint** area displays the preview schema size in terms of number of fields of view (FOVs). Additionally, the type of camera and objective used for preview is shown.

In the **Label Position** area, you can specify how your slide is oriented on the stage. This is not a definitive setting. For instance, even if your slides have the labels oriented down, you can change this later in the project for individual slides or for multiple slides. To do this later, just right click on the slide(s) and change its label position.

5.1.5 Finish: Experiment Ready

After you have defined your experiment settings, your experiment is ready to be created. The **Finish: Experiment Ready** window allows you to review a summary of your settings before proceeding.



- **Changes** can be made by simply clicking the ***<<Back*** button (

) and returning at the previous pages in order to modify the settings.

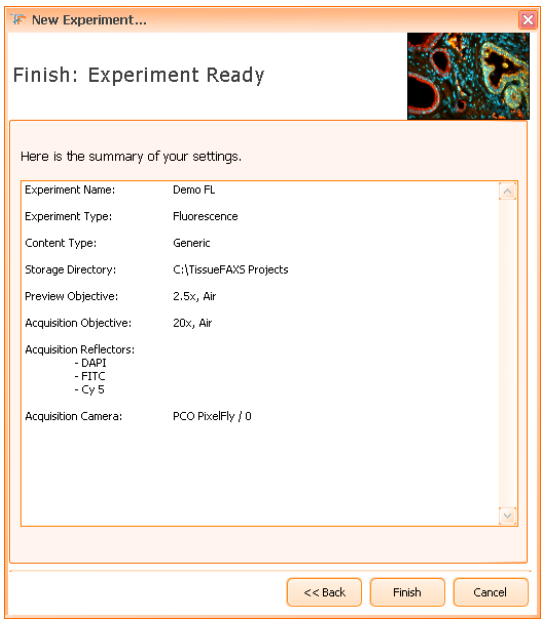


Figure 78 - Last page from wizard when creating a fluorescence experiment

5.2. Preview Settings

Preview Settings can be accessed by clicking on the **Preview** tab in the upper left corner of the main **TissueFAXS** window. If the **Preview** tab is not visible, you can make it visible by pressing the **Settings** button from the application's toolbar.

Figure 79 - Preview tab

This feature of **TissueFAXS** covers the settings for the preview operation, such as the objective (which must be a low magnification objective), channels (for instance the reflectors in use) and individual settings for each channel.

In the next two sections, the workflow for brightfield and fluorescence experiments will be described.

5.2.1 Brightfield Experiment

For a **Brightfield Experiment** you can select the objective used for preview.

To edit the camera settings for a channel, click the **View** button (

). For further details see **Chapter 5.7**. from the current manual.

Note

- You cannot add or remove channels. The default channel is **Transmission** and cannot be changed. If you don't have a **Transmission** channel (e.g. a missing reflector in the reflector changer) you will not be able to acquire brightfield images.
- To adjust the camera settings for the channel **Transmission**, you must have a camera present in your project. Otherwise, the **View** button (View) for each channel will not be visible.

CAUTION

- If you had made previews without any experiment and then created a new experiment, the previews will be lost.

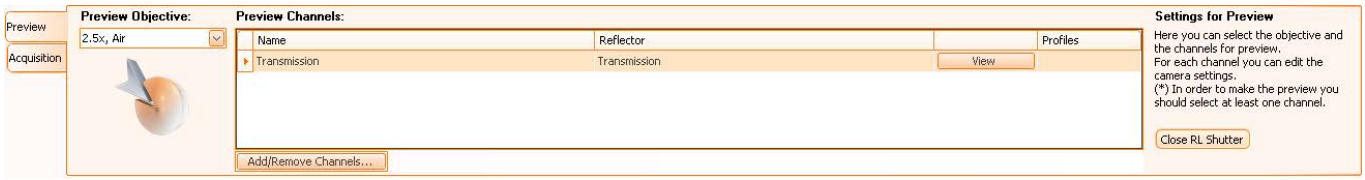


Figure 80 - Preview settings panel

5.2.2 Fluorescence Experiment

For a **Fluorescence Experiment**, you can select the objective used for preview, the channels used for preview and the camera settings for each channel.

To add or remove channels, press the **Add/Remove Channels…** button (Add/Remove Channels...). In the dropdown list that appears, the already selected channels will appear checked.

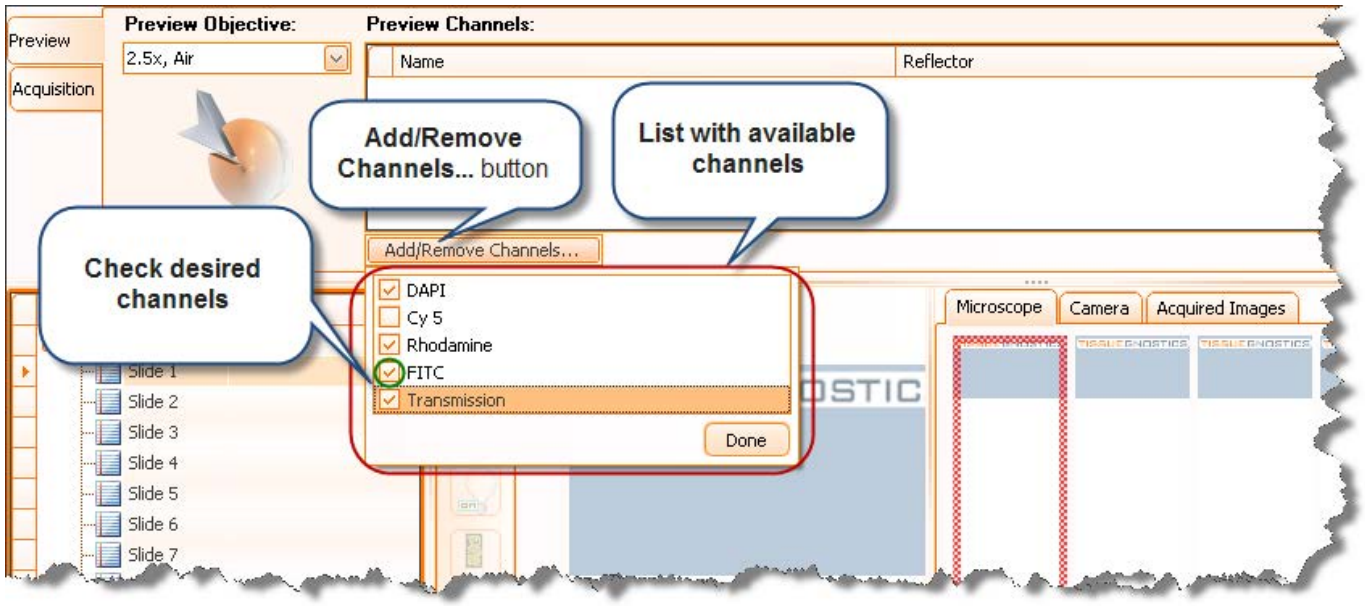


Figure 81 - Preview Settings panel: Channel list

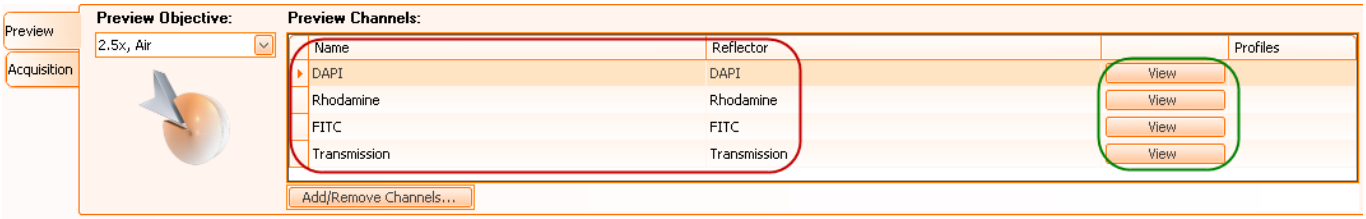


Figure 82 - Preview Settings panel: Channels inserted

To edit the camera settings for a channel, click the **View** button next to it. For further details see **Chapter 5.7**. of the current manual.

You can change the names of the channels by clicking on each in the settings field.

Note

- To adjust the camera settings for any channel, you must have a camera present in your project. Otherwise, the **View** button (View) for each channel will not be visible.

You can select more than one channel for the preview operation because each slide can be scanned multiple times (once for each channel in the list, that will yield the final overlay image). For each channel you may adjust the camera settings (light intensity, exposure time, colors, etc) by pressing the **View** button next to each reflector in the list. Make your adjustments in the new window that appears and press **Save**. When all your channel settings are saved, you are ready to acquire your preview image.

It might be an option to make the preview for fluorescent experiments in darkfield illumination.

5.3. Acquisition Settings

Acquisition settings can be accessed by clicking on the **Acquisition** tab in the upper left corner of the main **TissueFAXS** window. If the **Acquisition** tab is not visible, you can make it visible by pressing the **Settings** button from the application's toolbar.

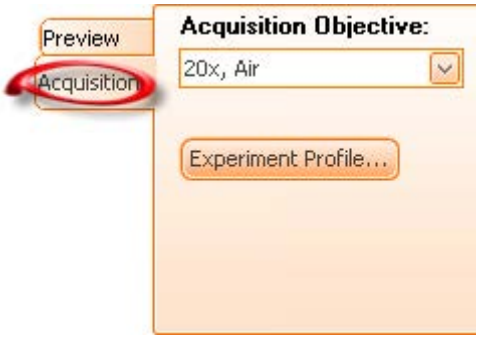


Figure 83 - Acquisition tab

TIP

- Acquisition settings are similar to the preview settings, except for the fact that they act when acquiring regions.
- The list of objectives which may be used for acquisition is not limited (as for the preview).
- An important difference is that you can select the channel for auto focus in the acquisition workflow.

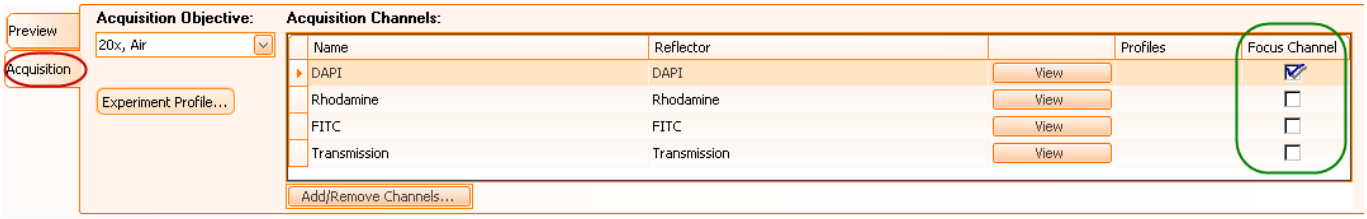


Figure 84 - Acquisition Settings panel

Focus Channel

The **Focus Channel** is the channel for which **TissueFAXS** will perform the auto focus in fluorescence experiments. The focus for **Focus Channel** will be memorized by the application and then used in acquiring the other channels.

The default **Focus Channel** in **TissueFAXS** fluorescence experiments is DAPI. If DAPI is absent from the experiment, **TissueFAXS** will automatically set as **Focus Channel** the first channel listed in the **Acquisition Channel** section.

Channels

- For Brightfield Experiment: Only **Transmission** is available;
- For Fluorescence Experiment: choose the desired reflector (including transmission).

Note

- After choosing the reflectors, you must readjust the camera settings for each reflector.
- The name of the channel is editable.

Objective Lens

Select the desired objective from **Acquisition** → **Acquisition Objective**.

Note

- After choosing the acquisition objective, you must readjust the camera settings for each combination of acquisition objective and acquisition channel.

Camera Settings

To edit camera settings press **View** button. For more information please see **Chapter 5.7** of the current manual.

You may also want to use settings from **intensity profiles** (if you have previously created any) instead of making new settings for the camera. For more information about intensity profiles please see **Chapter 5.9**.

Additionally, **TissueFAXS** stores TL lamp intensity value (for Transmission) and attenuator settings value (for Fluorescence channels) per channel.

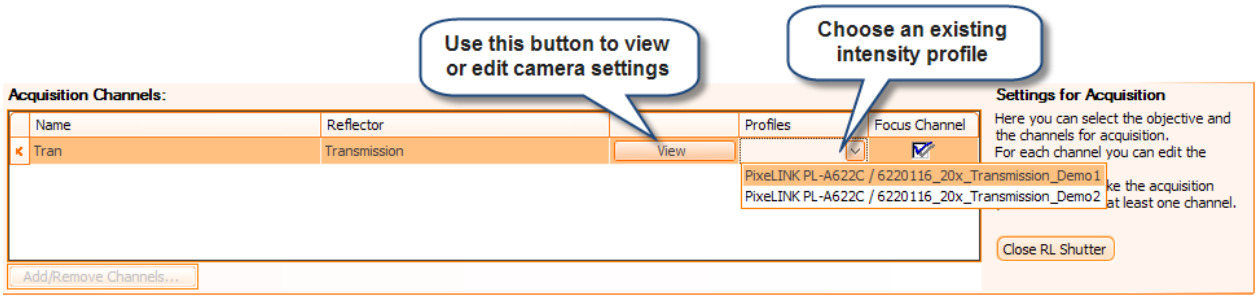


Figure 85 - Camera Settings

Experiment Profile

An **Experiment Profile** represents a set of settings (camera, acquisition objective, preview channels, acquisition channels) that you can save for further use, in order to make available different combinations of settings for the preview and acquisition. To access **Experiment Profile** options, go to acquisition settings tab and press **Experiment Profile** button…

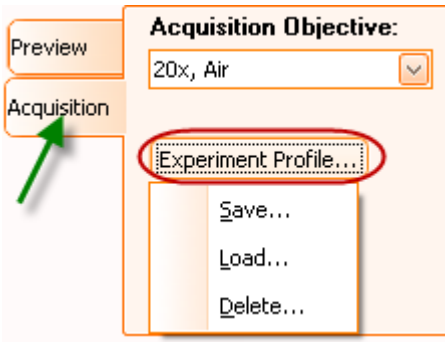


Figure 86 - Profile Options menu

- **Save**: the current preview and acquisition settings can be saved in order to make them available for other experiments. You must specify a name and a short description for this profile. A default name is already generated. It contains the camera name and type, the objective magnification and the reflectors selected for acquisition; you can also add your own information to the name.

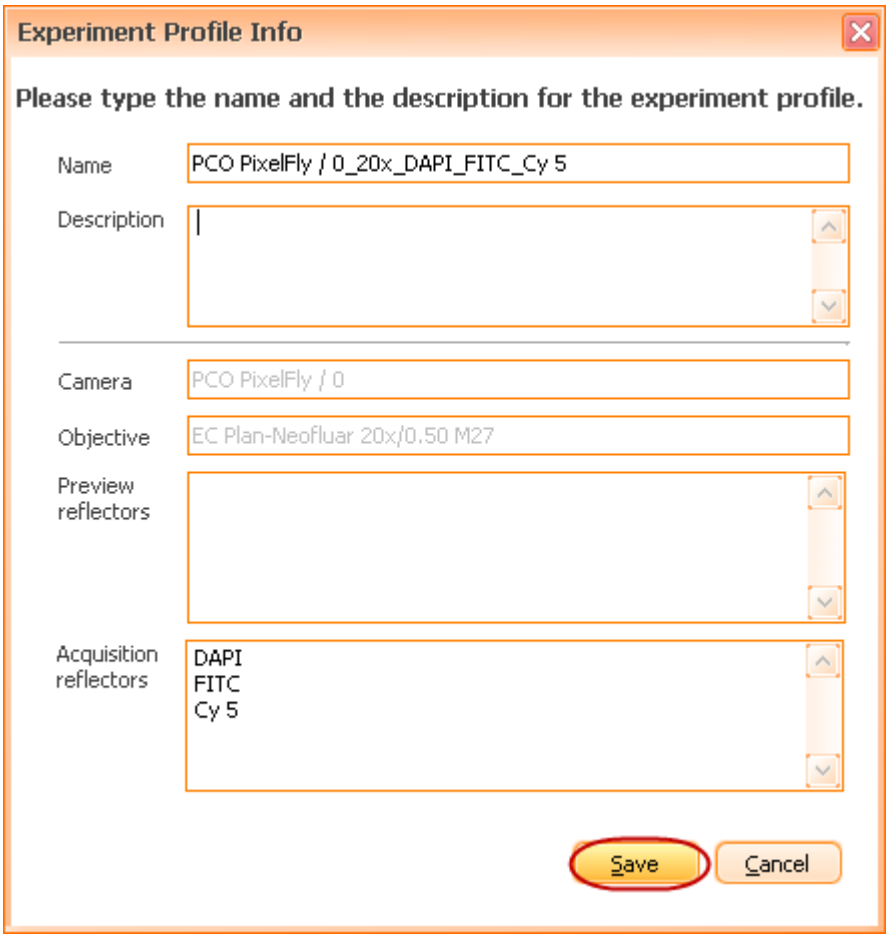


Figure 87 - Save Profile dialog

- **Load**: an existing profile can be loaded for current experiment. You must select any profile you want from the existing list.

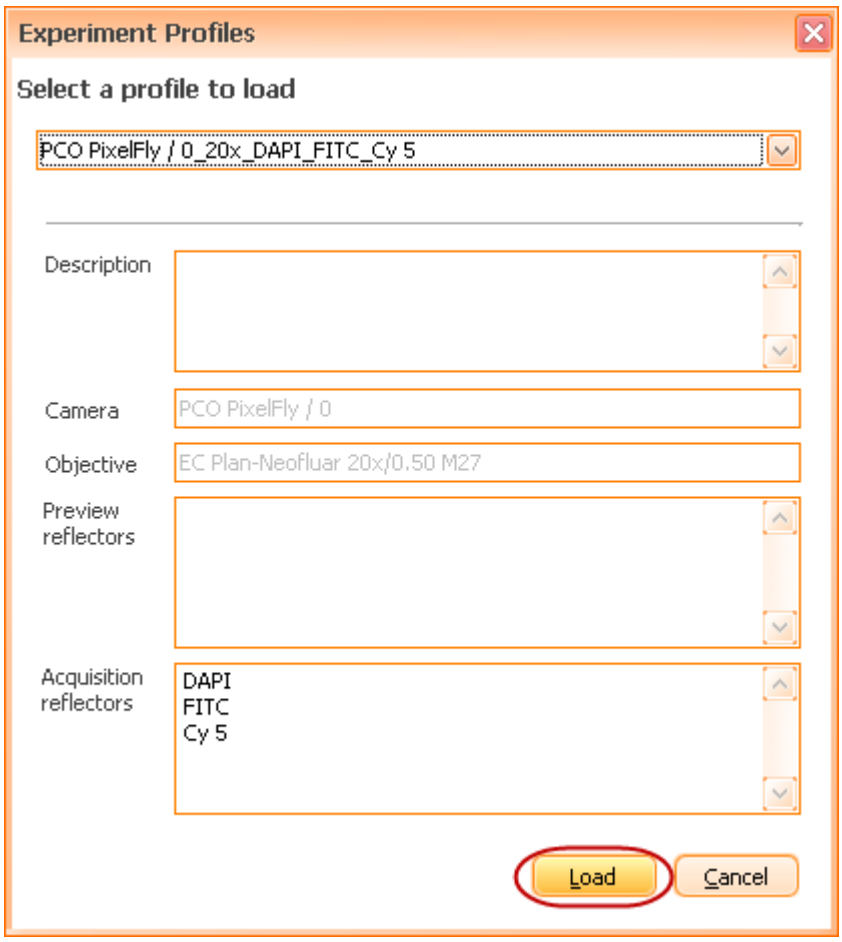


Figure 88 - Load Profile dialog

- **Delete**: if an existing intensity profile is no longer needed, it can be deleted by selecting it and then pressing the **Delete** button.

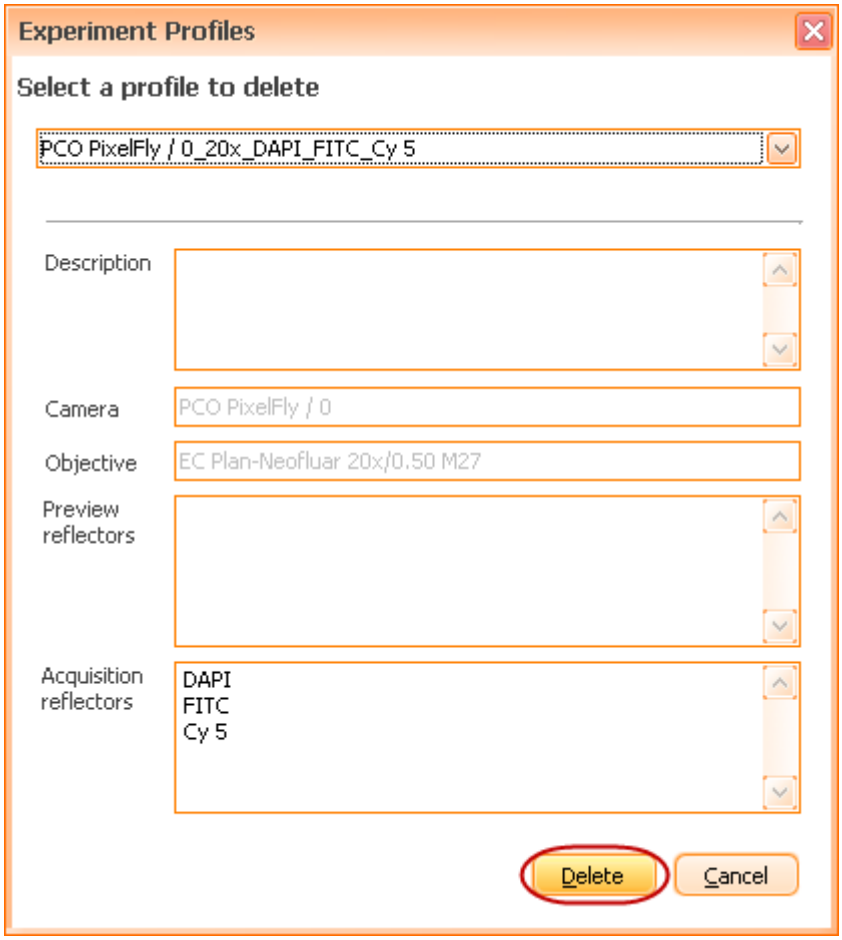


Figure 89 - Delete Profile dialog

Estimation of acquisition duration

TissueFAXS can estimate how long the acquisition process will take. This estimation is pretty rough at the first fields of view, but after at least two focus operations its accuracy will increase.

This feature is useful in case of unattended acquisitions and it is available in the bar from the lower-left corner of the application.

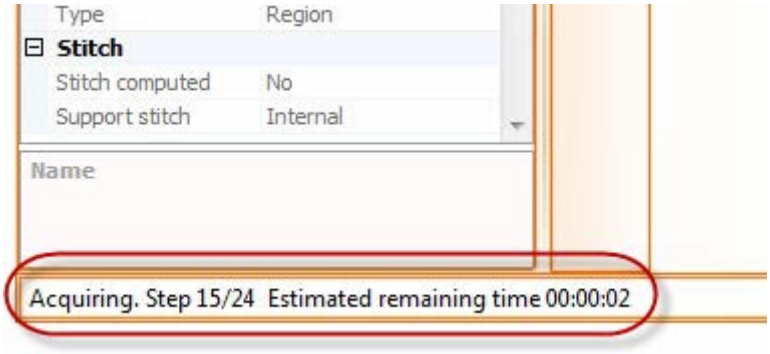


Figure 90 - Estimation of acquisition duration

5.4. Standard acquisition vs Time Lapse acquisition

Time lapse acquisition consists of multiple acquisitions for a region or a set of regions. The multiple acquisitions will be performed after a certain time interval specified by the user.

Time lapse feature is ideal for observing cell cultures.

How to acquire

A region can be acquired in **two modes**: **Standard** (normal) or **Time Lapse**.

You can specify the acquisition type in the **Acquisition Information** dialog that is displayed before the actual acquisition process is started.

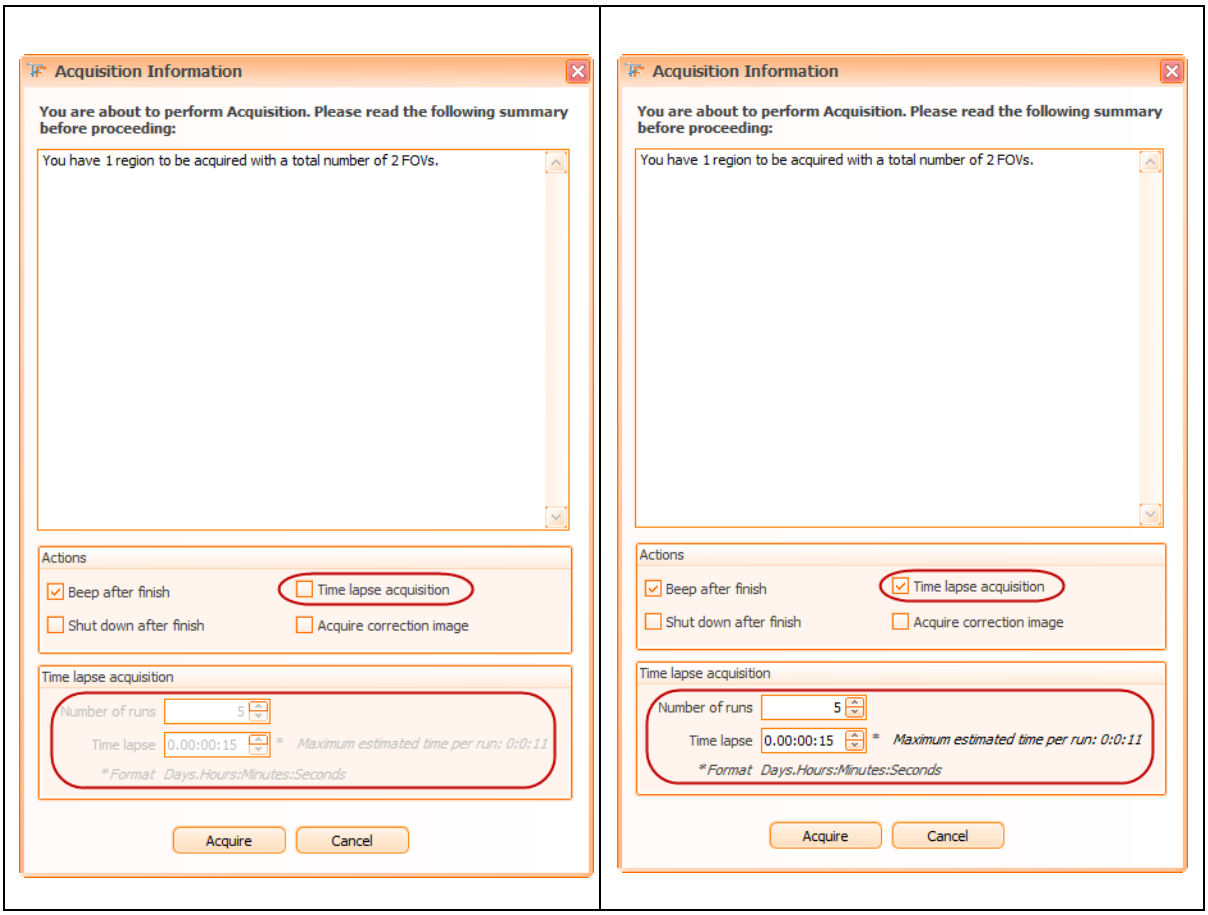


Figure 91 - Acquisition Information: Standard (left image) and Time Lapse (right image)

To enable **Standard acquisition** mode, the **Time Lapse acquisition** checkbox must not be ticked. To enable **Time Lapse Acquisition**, then **Time Lapse acquisition** checkbox must be ticked and the user should also set the values for the following parameters:

- *Number of runs*: how many times the acquisition will run;
- *Time lapse*: the interval between runs. The time lapse will be specified like this: *days.hours: minutes:seconds*.

Maximum estimated time per run

This message is displayed on the right side of **Time Lapse acquisition** section. It gives you an estimation of the time interval required for a run (maximum required time estimation).

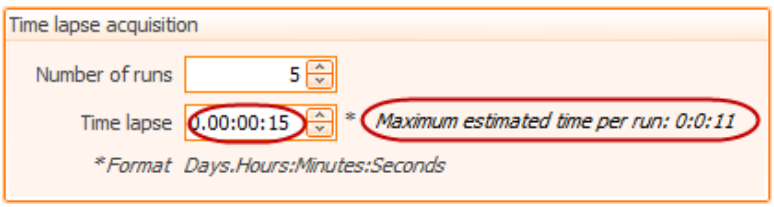


Figure 92 - *Maximum estimated time per run* message

If the time interval set in the **Time Lapse** field is too short to acquire the item, the message will be displayed in red.

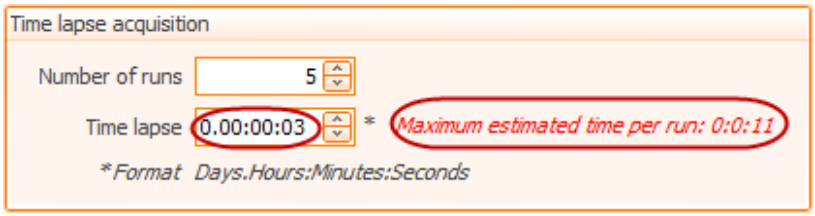


Figure 93 - *Maximum estimated time per run*: low time lapse value

In this case, by pressing **Acquire** button, a message box will inform you again abut the short time lapse.

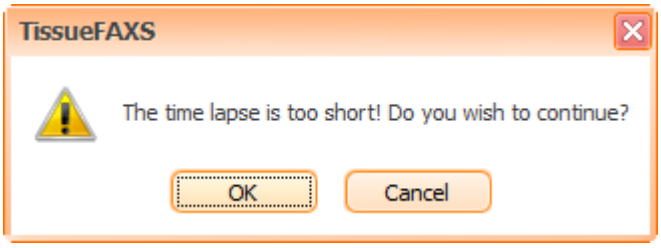


Figure 94 - Short time lapse message box

If you continue with the acquisition process despite the short time lapse, the acquisition process will start and a warning message will be displayed in the status bar located in the lower part of the application: *"Time lapse acquisition overlaps!"*

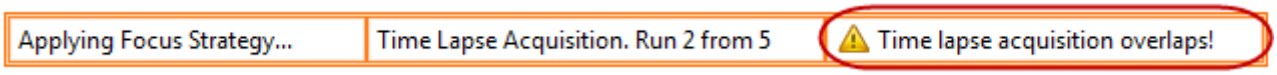


Figure 95 - Acquiring with short time lapse: warning from status bar

For every region acquired with short time lapse, this information will be displayed in the **Region properties** section from the **Experiment Editor (**see Chapter 5.14).

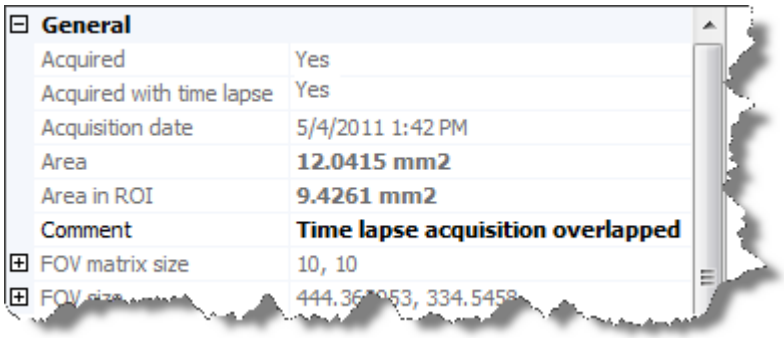


Figure 96 - Experiment editor: region acquired with short time lapse

TIP

- If you frequently use **Time Lapse** acquisition, you can set this option as default from **Tools → Options → Application Options → Remember** (along with the desired parameters values).

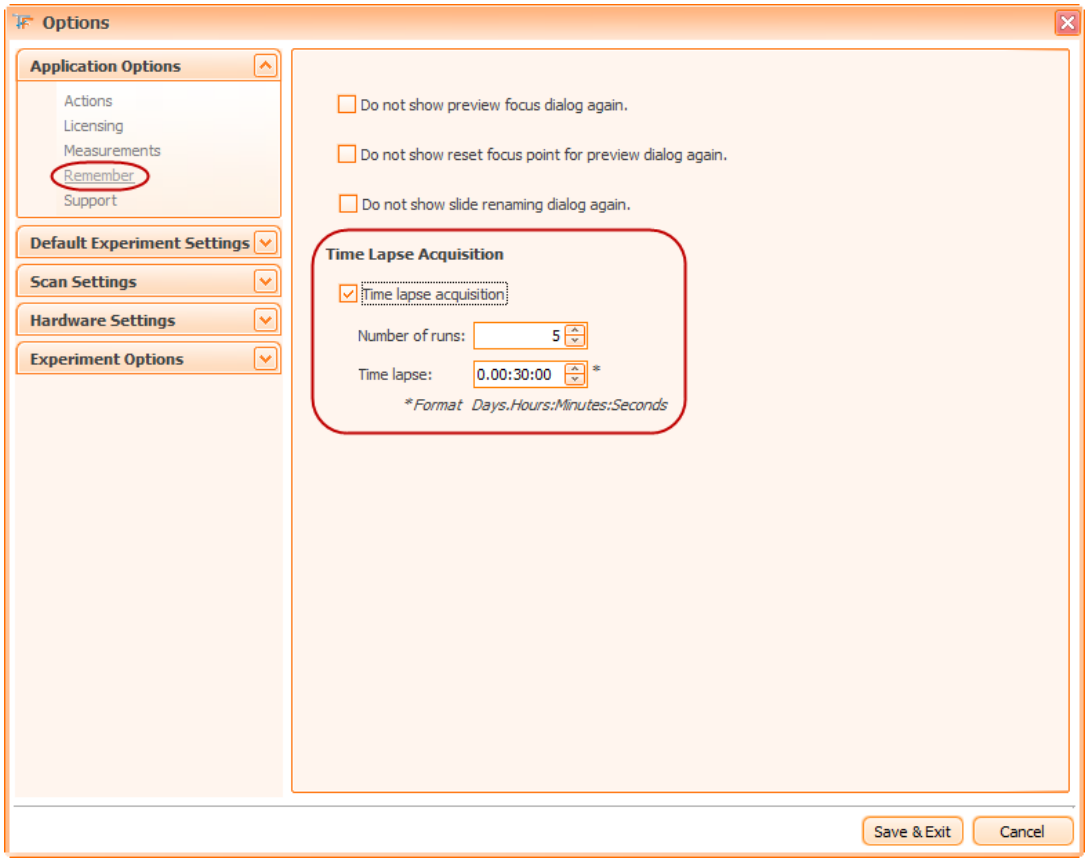


Figure 97 - Remember dialog: Time lapse acquisition section

How to view

By default, when double clicking on a region in **Experiment Editor**, that region will open in **Time Lapse** mode (if time lapse images have been acquired) or in **Standard Mode** (if time lapse images have not been acquired).

Be aware that not all operations normally available on a region are available on a set of **Time Lapse** images (see also Chapter 5.17):

- Adding/Removing FOVs option is not available;
- Reacquisition of specified FOVs is not available;
- Resize of the region is not available;
- There is only one correction image for all Time Lapse images belonging to the same region;
- All the categories/subregions will be the same for all Time Lapse images belonging to the same region.

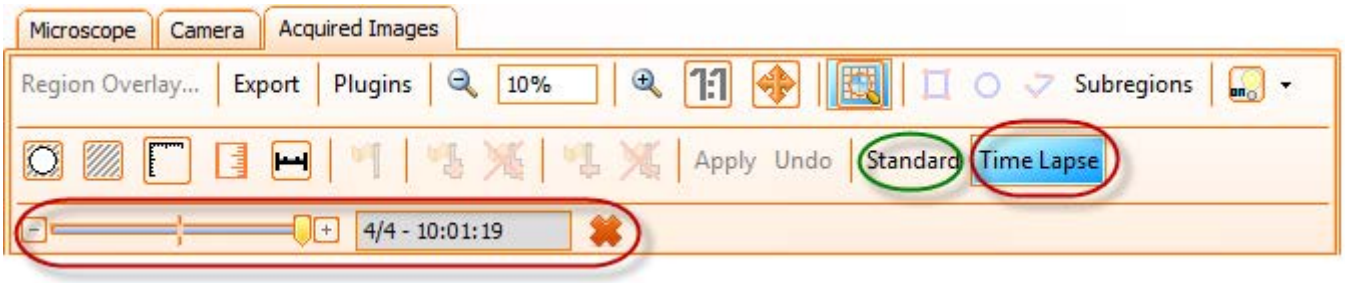
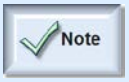


Figure 98 - Region viewer toolbar: Time Lapse mode

Assuming a region has both **Standard** and **Time Lapse** images, the user can switch between these two modes by using two buttons: Standard ↔ Time Lapse.

The user can choose to see the region at a certain moment by moving the cursor ( ) or by pressing and buttons.

The user can delete all time lapse images by pressing the **Remove** button ( ).



- **Time Lapse acquisition** is not available for TMA samples (see Chapter 6).
- During the **Time lapse acquisition** process, the user can see the **progress of the acquisition** in the status bar of the application.

Figure 99 - Example of Status Bar

5.5. Microscope

In this section of the manual, you will find a description of those components of the microscope that can be controlled by using **TissueFAXS** and you will learn how to use them.

5.5.1 Objectives

Figure 100 - Objective Turret control (a)

The **Objectives** panel displays the objectives available in the microscope's **Objective Turret**.

Note The first listed missing objective is displayed as **0x**. The rest of the missing objectives are not displayed.

Figure 101 - Objective Turret control (b)

Select an objective from this panel by clicking on it. This will switch the current objective to the selected one.

Note The objective in use is always shown in red.

5.5.2 Reflectors

Figure 102 - Reflector Turret control (a)

The **Reflectors** panel displays all the reflectors available in the microscope's **Reflector Turret**.

Note The first listed empty position in the reflector turret is displayed as **Transmission**. The rest of the missing reflectors are not displayed.

Figure 103 - Reflector Turret control (b)

Select a reflector from this panel by clicking on it. This will switch the current reflector to the selected one.

Note The current reflector in use is always surrounded by a black rectangle.

Edit Reflectors

A good management of your reflectors will provide good quality acquired images and will also help you later in your image analysis.

Right clicking on a selected reflector will show a menu with two options:

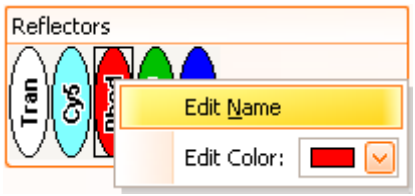


Figure 104 - Context menu for selected reflector

- **Edit Name** - the user can change the **Full Name**, the **Short Name** and the **Abbreviation** (max. 4 characters) for the selected reflector. Also, the user can write a short description for this reflector.

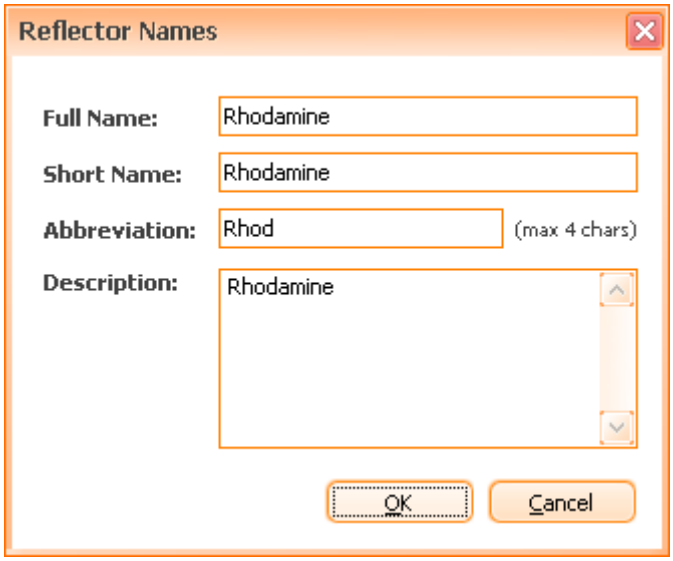


Figure 105 - Edit details for selected reflector

- The user also has the possibility to choose the displayed names for the reflectors in the application by going to **Tools** → **Options…** → **Reflectors**.

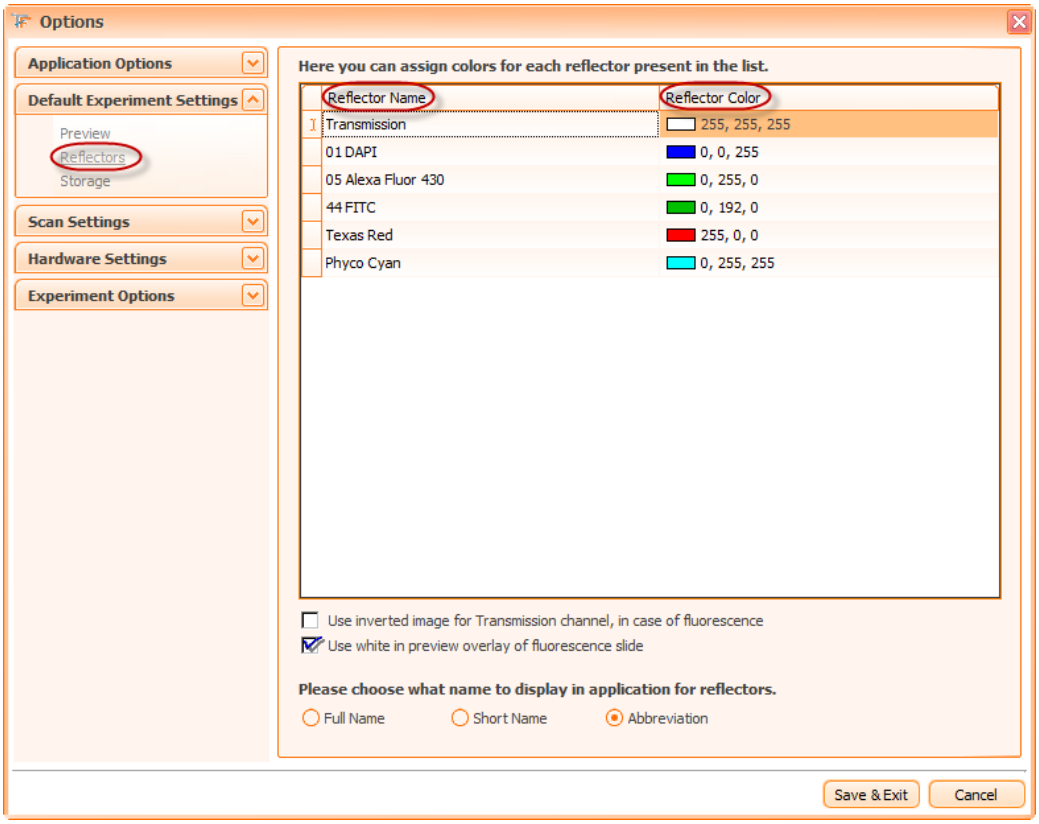


Figure 106 - Reflector Settings dialog

- **Edit Color** - the user can set the color for the selected reflector.

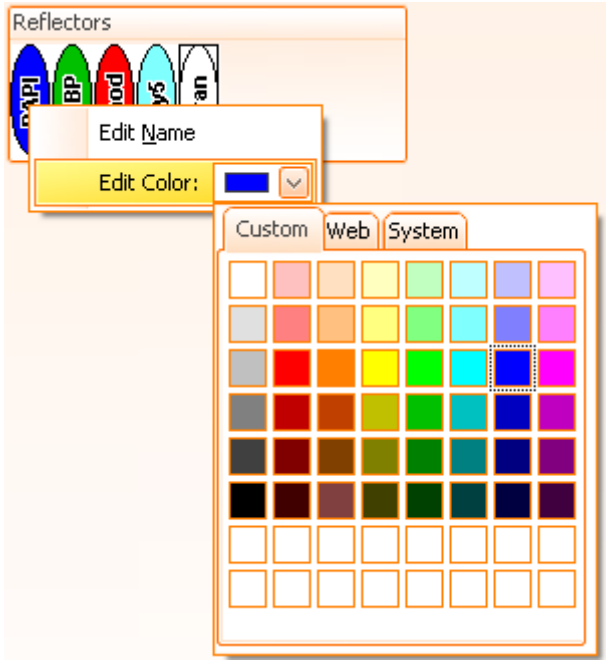


Figure 107 - Edit Reflector Color context menu

TIP - When the mouse is over a reflector, an information dialog is automatically displayed. It contains brief information as **abbreviation**, **full name** and **description**.

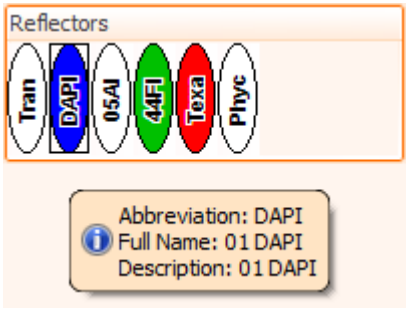


Figure 108 - Reflector Info bubble

5.5.3 TL Shutter for transmitted light

TL Shutter is used to block or unblock the light coming from the halogen or white-light LED lamp.

Figure 109 - TL Shutter control (a)

The **TL Shutter** is closed.

Click on the shutter icon to open the TL shutter.

Figure 110 - TL Shutter control (b)

The **TL Shutter** is opened.

5.5.4 TL Lamp

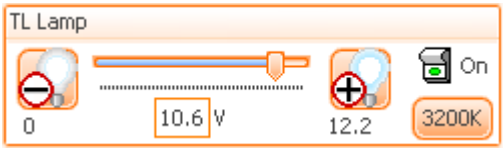


Figure 111 - TL Halogen Lamp control

Use the light switch icon to turn on/off the TL Lamp. Right to the switch, the current state of the **TL Lamp** is displayed (**On/Off**).

Check the 3200K check box to turn on the color temperature of the TL Lamp directly to 3200K.

Note The color temperature can only be set for halogen lamps. This checkbox has no relevance for white-light LED.

Adjust the light intensity by moving the slider.

Note - There is a small delay between changing a value on the control and the actual response of the lamp. For instance, when the lamp is turned on, it takes some time until it reaches the luminosity corresponding to the control value.

5.5.5 RL Shutter for reflected light

RL Shutter is used to block or unblock the light coming from RL lamp. This way, the light maintains the same intensity compared to on/off cycle and the lifetime of the lamp is increased.

Figure 112 - RL Shutter control (a)

RL Shutter is closed.

Click the RL shutter icon to open the RL shutter.

Figure 113 - RL Shutter control (b)

RL Shutter is opened.

5.5.6 Fluo Arc

A **Fluo arc** is a device used to dim the fluorescent lamp. It should be run at 100 % or it will flicker and give bad results.

Figure 114 - Fluo Arc control

Fluo Arc control when present.

5.5.7 White Light Attenuator (optional component)

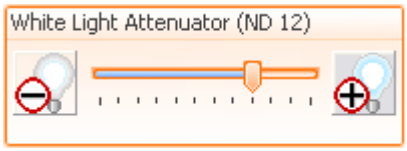


Figure 115 - White Light Attenuator control

Adjusts the transmission light intensity using some filters.

5.5.8 Fluorescence Attenuator (optional component)

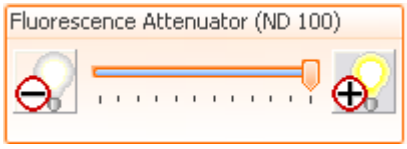


Figure 116 - Fluorescence Attenuator control

Adjusts the reflected light intensity using some filters.

5.5.9 Condenser

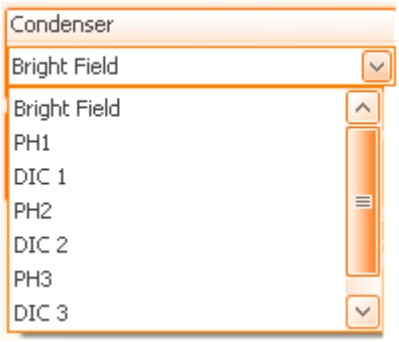
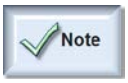
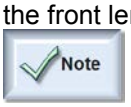


Figure 117 - Condenser combo box

The **Condenser** combo box allows you to select the desired contrast method.



In fluorescence, it is recommended to move the front lens out of the lightpath of the condenser.



Phase Contrast (PH) and Differential Interference Contrast (DIC) are optional components.

5.5.10 Condenser Front Lens

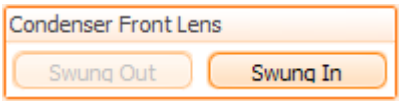


Figure 118 - Condenser Front Lens

Using the control, you can swing in or swing out the condenser front lens.
For objective lenses with magnification factors smaller than or equal to 10x and during fluorescence acquisition, the front lens must always be swung out. Only for objective lens magnification factors higher than 10x in transmitted light, the front lens is needed for concentrating the light onto the field of view.

5.5.11 Condenser Aperture

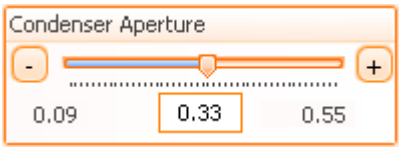


Figure 119 - Condenser Aperture control

Using the slider, you can open or close the aperture diaphragm.

The condenser aperture determines the contrast in the image. Wrong contrast settings might impose autofocus functionality and the quality of image analysis. As a good start point, the condenser aperture should be close to the numerical aperture of the objective lens in use. The Light Manager will store individual settings for each objective lens installed.

5.5.12 Sideport Changer

Figure 120 - Sideport Changer combo box

Sideport Changer control when present.

It is used to specify to which port (observation, rear port, front port) the light is directed. Thus, the user can switch between visual observation through the oculars and the digital live image through a camera.

5.6. Stage

Stage Components

The stage is the hardware component of the microscope that allows viewing different inserts and their contents (tissue, cell cultures, etc), by moving on the X/Y axes. **TissueFAXS** provides a virtual image of the stage with its existing inserts.

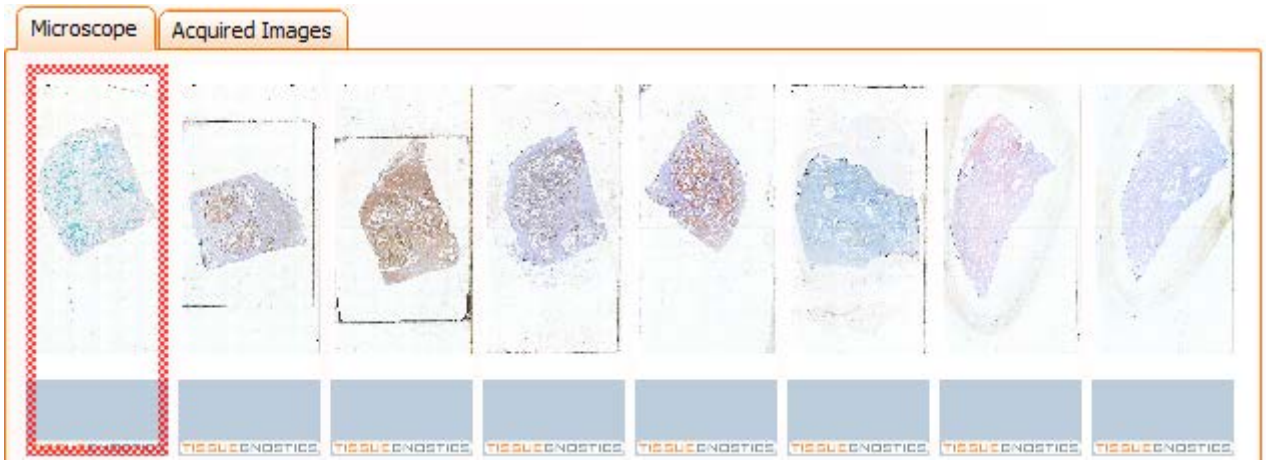


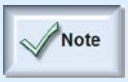
Figure *121* - Stage Control

The currently selected slide can be recognized by a surrounding red frame.

Figure 122 - Slide selected by the user

The slide currently placed under the objective lens can be recognized by a surrounding green frame.

Figure 123 - Active Slide on the microscope



- For more information, please see **Chapter 5.15** of the current manual.

5.7. Cameras

TissueFAXS supports the following cameras:

- PixeLINK cameras: select this type of camera for **Brightfield** imaging;
- PCO PixelFly cameras: select this type of camera for **Fluorescent** imaging.

5.7.1 Adding Cameras

To add a supported camera, follow the steps mentioned below:

- From the **Tools** menu select **Options…** to open the **Options** dialog box.
- In the **Options** dialog choose **Hardware Settings** → **Camera Manager**.

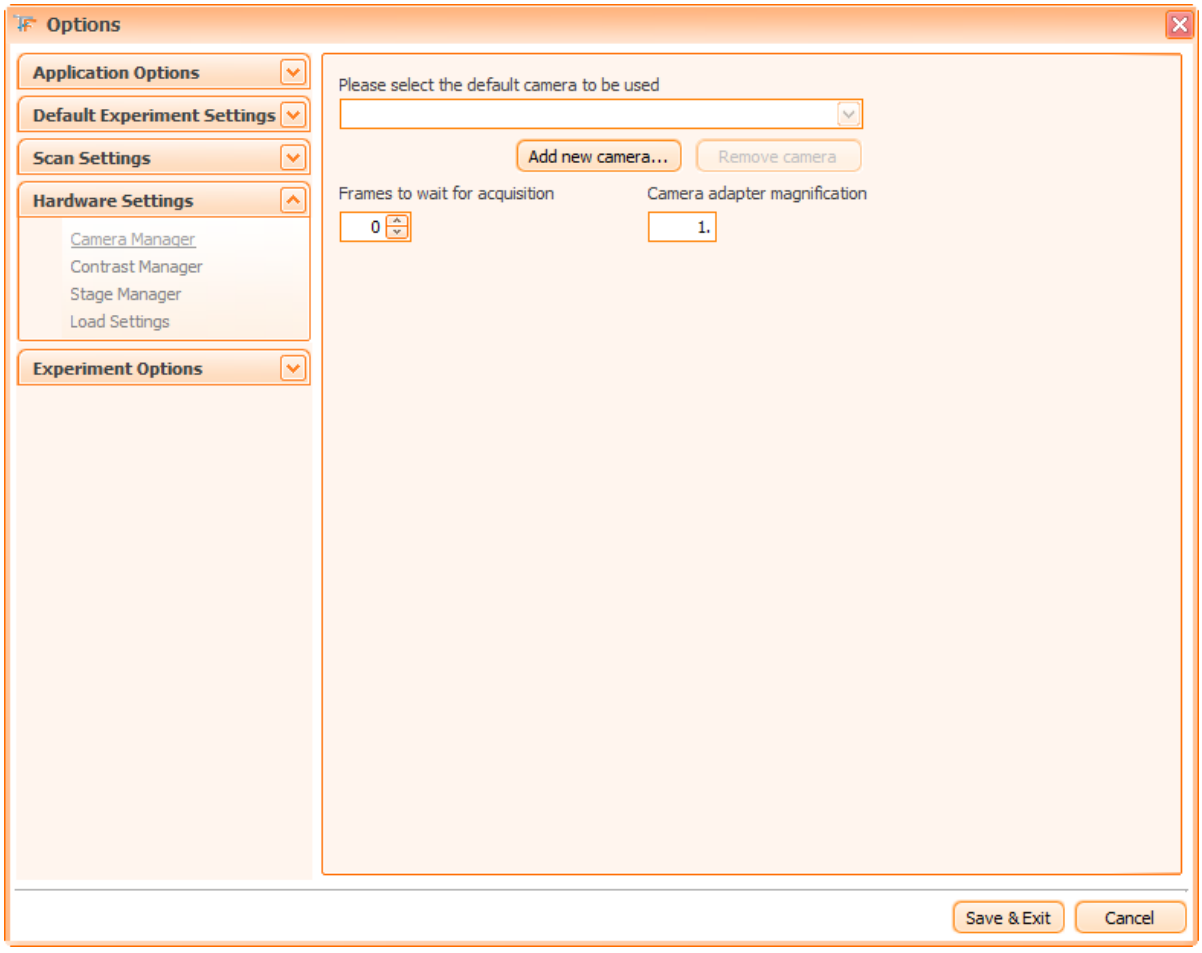


Figure 124 - Camera Manager dialog

- Press **Add new camera…** button. The **System Cameras** dialog box will appear and it will display the cameras that are supported by **TissueFAXS** and are connected to your computer.

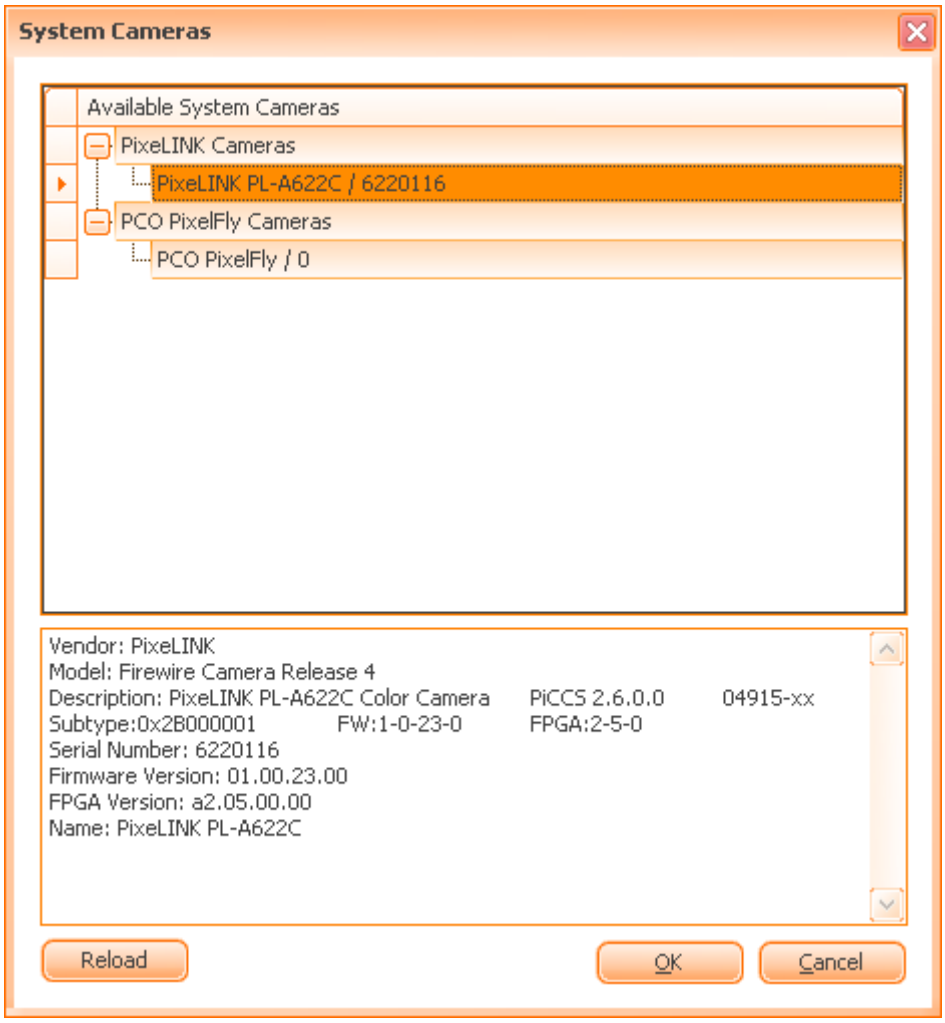


Figure 125 - System Cameras dialog

- In the **System Cameras** dialog select the desired camera and press **OK**. If your camera is not listed there, you can press **Reload** to rescan for connected cameras.

- If your camera is already used in **TissueFAXS**, it is possible not to be listed, even if you press **Reload**.

- Now, you will be able to see the selected camera in the combo box.
- **Frames to wait**: This value indicates how many images the camera will skip before the image will be taken and stored. If this value is too low, the images may appear stretched, because they were taken before the stage has completely stopped after rapid movement (motion blur). Default values are 1 or 2; higher values may be suitable if you are working with cell culture dishes.
- **Camera adapter magnification**: this is an adapter used to connect the camera to the microscope, having its own magnification factor (written on it); if this information is missing or is wrong in **TissueFAXS**, the total magnification of the experiment will be wrong;
- Press **Save & Exit** to save the settings and close the **Options** dialog.

5.7.2 Change Default Camera

You can change the camera you are using any time you need. To perform this operation follow the steps described below:

- From the **Tools** menu select **Options…** to open the **Options** dialog box;
- In the **Options** dialog choose **Hardware Settings** → **Camera Manager**;
- Select the desired camera from the combo box. If the camera is not listed there, please see the steps described in **Chapter 5.7.1.**
- Press **Save & Exit** to save the settings and close the **Options** dialog.

5.7.3 Operations with Current Camera

The camera you are currently using allows you the following actions:

- Enable or disable the live image for the current camera (depending on what your are working at, you may want to see the live image or not);
- Capture an image with the current camera (the main purpose when using the camera);
- Adjust the settings for the current camera (in order to obtain the desired results – in preview or image acquisition – you are able to constantly adjust your camera settings).

5.7.4 Remove Camera

You can remove the camera you are using any time you need. To perform this operation follow the steps described below:

- From the **Tools** menu select **Options…** to open the **Options** dialog box;
- In the Options dialog choose **Hardware Settings** → **Camera Manager**;
- Select the camera you want to remove from the combo box and press **Remove camera** button.

- If the camera is not present in the combo box, its removal is no more necessary.

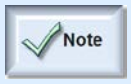
5.7.5 Settings for PCO/PixelFly Camera

The settings available for both cameras are divided in two categories: basic settings and advanced settings. Basic settings are the most frequently used, while advanced settings are rarely used, usually when the system is installed or in special conditions (as described in sections below).

- Changes in the Advanced Settings menu should only be done by informed users, understanding the relevance of these controls. Changing these parameters in an unqualified way may cause **TissueFAXS** to malfunction.

Basic Settings

Exposure Time: controls the amount of time available to the sensor for collecting light to form an image. In Assync Shutter Mode (low brightness) the exposure time range is 0.01…65 ms. In Video Mode (high brightness) the exposure time range is 1…10000 ms. Increase this parameter when the illumination condition are poor or when the markers on the samples are dim (in fluorescence experiments).



- Due to the large value (10000) allowed for exposure time, this control starts with the maximum value of 500 in order to increase usability. If you require higher exposure time, do a right click on this control and access the **Maximum** menu.

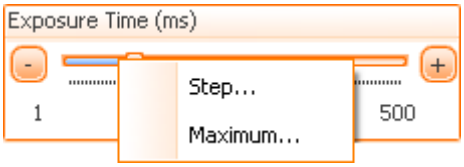


Figure 126 - Exposure Time control

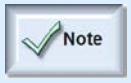
Figure 97 - Changing the maximum exposure time allowed combo box

Gain: Controls the intensity of the image by artificially increasing the values of pixels by using software multiplication. Use this when you don't want to increase the exposure time, in order to increase the acquisition speed.

Figure 127 - Gain control

Lower / Upper: These parameters specify the interval which will be scaled to 256 gray levels for display.

- **Lower Sensitivity Threshold**: by increasing this, the low intensities (like background) in the image will be lost.
- **Upper Sensitivity Threshold**: by decreasing this value, the shades in the image will become brighter.



- If you want to manually adjust these parameters, you should set them in such a way that the lower parameter limit equals the smallest value for which the "High background" is green and the highest value for the upper parameter for which the "Saturation" indicator is green.

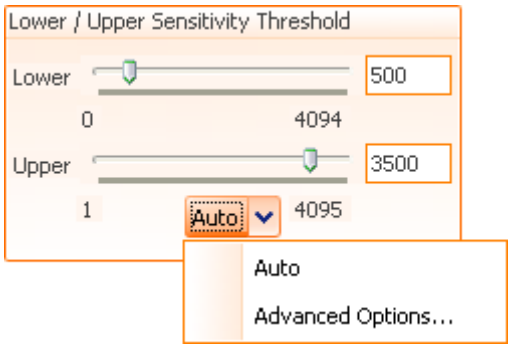


Figure 128 - Lower / Upper parameters controls

You can automatically adjust these parameters by pressing the **Auto** button.

By choosing **Advanced Options…** the following dialog will appear:

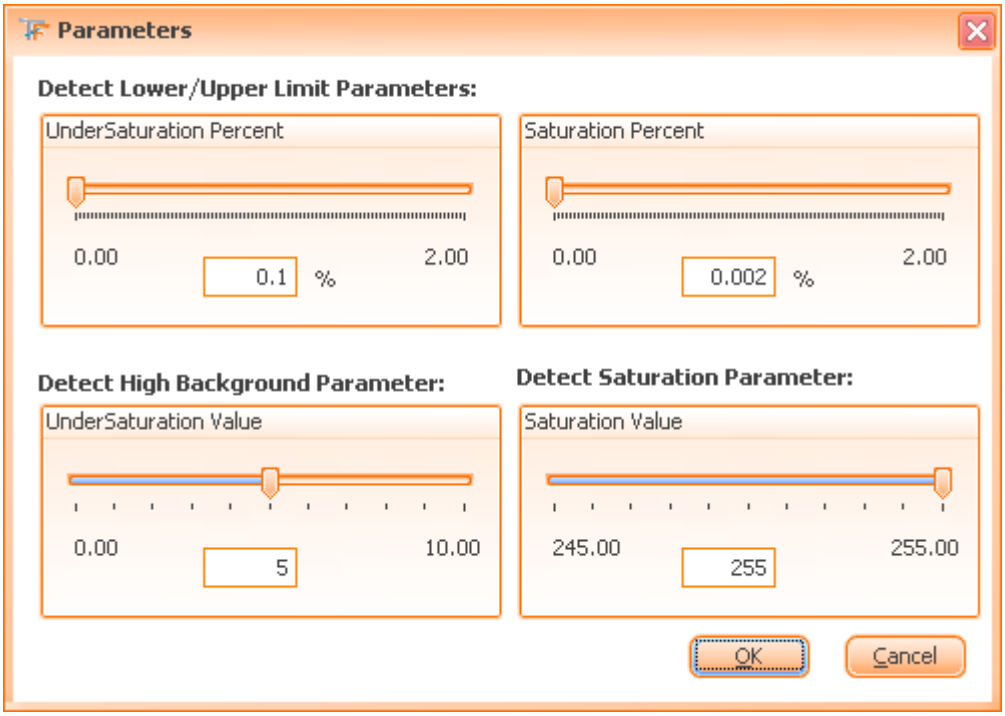


Figure 129 - Options for detect Lower / Upper Limit Parameters control panel

The advanced options control how **TissueFAXS** detects the presence of oversaturation and undersaturation (lack of signal) in the camera image.

UnderSaturation Percent: If the number of pixels with intensity value higher than the (under-) saturation limit is over this percent of the total number of pixel then the image is considered undersaturated.

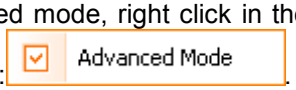
Saturation Percent: If the number of pixels with intensity value higher than the (over-) saturation limit is over this percent of the total number of pixel then the image is considered oversaturated.

UnderSaturation Value: The maximum acceptable intensity value for witch a pixel is considered undersaturated.

Saturation Value: The minimum acceptable intensity value for which a pixel is considered oversaturated.

Advanced Settings

In order to access the advanced mode, right click in the area where the camera parameters are.

The following menu will appear: 

. The **Advanced** tab will appear if checked.

Pixel Addressing: this parameter is used in order to decrease the size of the captured image and decrease the time needed for the capture operation. This will increase overall acquisition speed, but the image quality will be affected (reduced).

Figure 130 - Pixel Addressing combo box

Flip: this parameter is used to flip the captured image. You can use this parameter to correct the orientation of the image, if the left/right or top/down of the camera are different that those of the stage. This parameter is generally adjusted when the system is installed. The FOV calibration feature is able to automatically set this parameter to its correct value. Do not change this setting unless you encounter failure in stitching the overview images.

Figure 131 - Flip for the Image checkbox

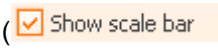
Camera Mode: You can choose **Async Shutter** or **Video Mode**.

- The **Async Shutter** is recommended when there is a high amount of light reaching the camera sensor (like in **Brightfield**). The stepsize of the exposure time can be adjusted in 1/10 of a second to a maximum exposure time of 50 ms.
- The **Video Mode** is recommended when there is a low amount of light reaching the camera sensor. This mode is recommended for **Fluorescence** experiments. The stepsize of the exposure time can be adjusted in 1-second steps to a maximum exposure time of 10,000 ms (10 sec).

Figure 132 - Camera Mode radio buttons

Display Information on Camera (

): when enabled, this option will display on the live image the histogram, the saturation and the high background indicators.

Display Scale bar (

): when enabled, this option will display the scale bar on the live image.

Display Gray value (

): when enabled, this option will display the gray value of a pixel selected with the mouse.

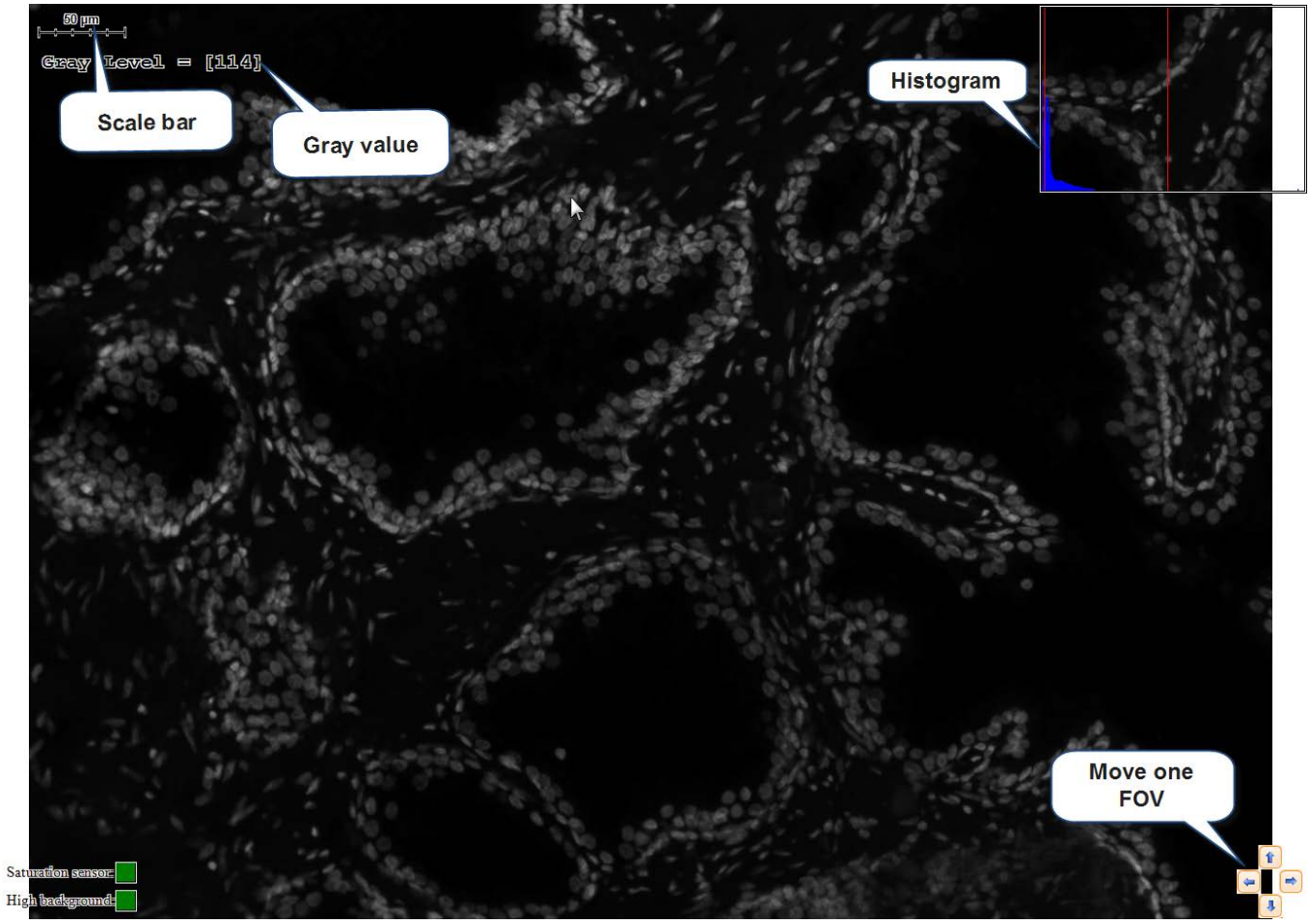


Figure 133 - Information Displayed on Camera

Note - If areas appear labeled in red within a fluorescence image, this means those areas are (over-)saturated.

5.7.6 Settings for PixeLINK Camera

The settings available for both cameras are divided in two categories: basic settings and advanced settings. Basing settings are the most frequently used, while advanced settings are rarely used and should only be changed by experienced users. Usually, advanced settings are only needed when the system is installed or in special conditions (as described in sections below).

Basic Settings

Exposure Time: The amount of time available to the sensor for collecting light to form an image. Increase this parameter when the light intensity is low.

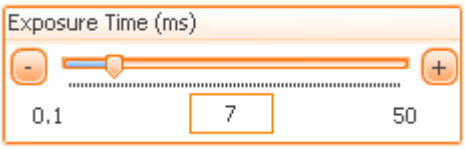


Figure 134 - Exposure Time control

White Balance: this parameter adjusts the intensity of the Red, Green and Blue colors in order to render a neutral color correctly (white is usually used as a reference). You can press **Auto** to autobalance the colors. When the transmitted light is not white, you can use this parameter in order to correct the hue of the light in order to obtain the reference white light.

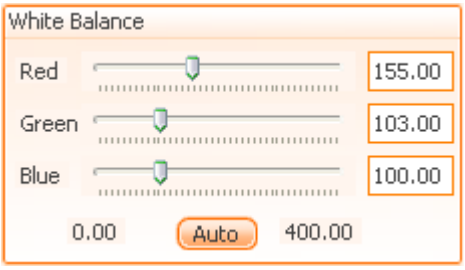


Figure 135 - White Balance control

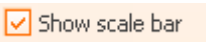
Saturation: represents the purity or strength of a color, due to absence of black, white or gray. Adjusting color saturation will affect how intense the tones of the color are in your image. Use this in order to enhance colors on tissues with faded colors.

The default value for **Saturation** is 150. If the user wants to set the default value for this parameter he must press the **Default** button.

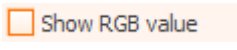
Figure 136 - Saturation control

Display Information on Camera (

): when enabled, this option will display on the live image the histogram, the saturation and the high background indicators.

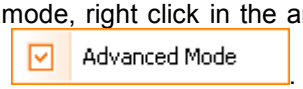
Display Scale bar (

): when enabled, this option will display the scale bar on the live image.

Display RGB value (

): when enabled, this option will display the RGB value on the live image.

Advanced Settings

In order to access the advanced mode, right click in the area where the camera parameters are. The following menu will appear: 

. The **Advanced** tab will appear when checked.

Frame rate: the maximum number of images captured per second.

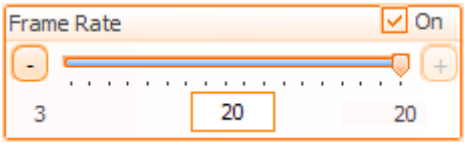


Figure 137 - Frame Rate control

Pixel Addressing: this parameter is used in order to decrease the size of the captured image and decrease the time needed for the capture operation. This will increase overall acquisition speed, but the image quality will be affected (reduced).

Figure 138 - Pixel Addressing combo box

Color Temperature: this parameter is similar to white balance making the picture look colder or warmer.

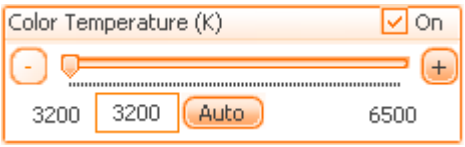


Figure 139 - Color Temperature control

Pixel Format: the format used when transferring images from the camera (only experts in imaging should change the settings).

Figure 140 - Pixel Format combo box

Resolution: this is the size (in pixels) of the captured images.

Figure 141 - Resolution combo box

Flip: this parameter is used to flip the captured image. You can use this parameter to correct the orientation of the image, if the left/right or top/down of the camera are different that those of the stage. This parameter is generally adjusted when the system is installed. The FOV calibration feature is able to automatically set this parameter to its correct value.

Figure 142 - Flip Image control

Rotate Image: this parameter is used to rotate the captured image. Use this when the camera is placed in a 90/180/270 degrees position in relation to the stage.

Figure 143 - Rotate Image radio buttons

Gain: artificially adjusts the intensity of pixels (by software multiplication of pixel values).

Figure 144 - Gain control

Sensor saturation: This set of parameters has no effect on the final images. This sensor is used only to warn the user of possibly over saturated pictures that can later affect the illumination correction process. The two parameters are:

- **Max allowed values**: if you have a very bright image that is considered oversaturated (the sensor turns red), you can increase this value in order to make the sensor accept it.
- **Sensor saturation area**: if the number of bright pixels (with a value above maximum allowed) is lower than Sensor saturation area, the image is considered to be fine; otherwise, it is considered oversaturated. Generally, you might want to increase this value when working with samples having many bright areas.

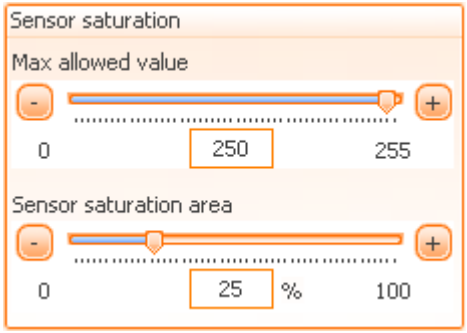


Figure 145 - Sensor saturation

Gamma: adjusts the contrast of the image. You can use this to highlight the differences between the light and dark areas or in order to minimize these differences.

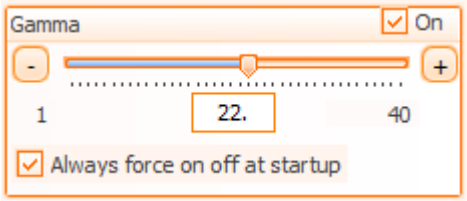


Figure 146 - Gamma control

5.8. Camera Panel

The **Camera Panel,** one of the essential tools in **TissueFAXS,** contains the stage control, the camera controls and a panel where controls for the microscope can be dragged. This panel containing controls for the microscope proves to be very useful, as it allows you to have ready at hand exactly the controls you need at the moment. If you want to remove the controls from the camera-window, drag them into the dustbin above. You will be able to drag them again from the microscope panel if needed later.

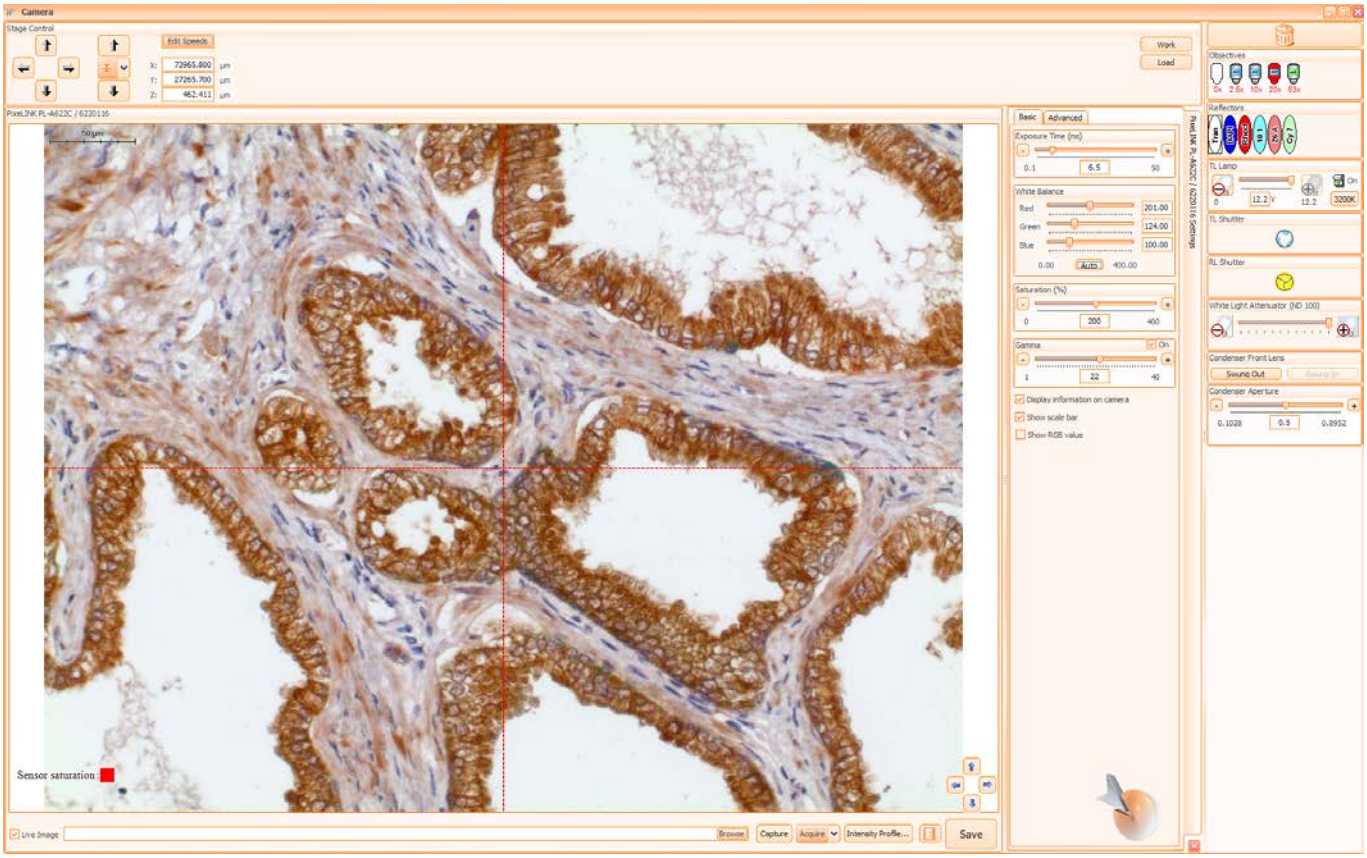


Figure 147 - Camera Panel

5.8.1 Stage control

This panel helps you to accurately control the movement of the microscope's stage.

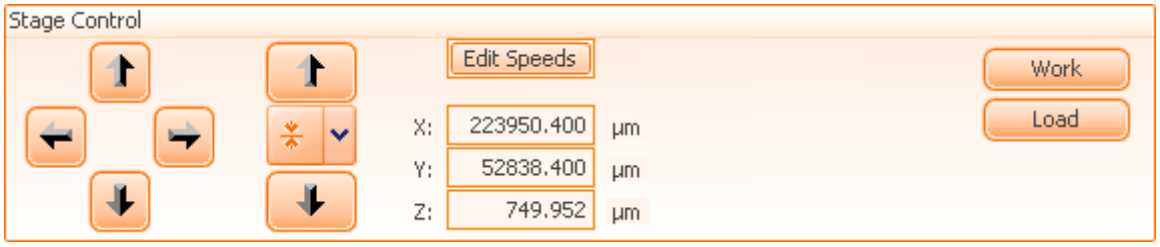


Figure 148 - Joystick control

Stage control components

- **Joystick control**: The four arrows of the Joystick control can be used to move the stage on the X and Y axis. The adjacent **Up** and **Down** buttons control the Z axis and are used to focus on the current field of view.

- **Autofocus button** ( ): by clicking this button, the microscope will autofocus on the current field of view.
- **Edit Speeds** (Edit Speeds): this button allows you to adjust the speed at which the stage is moving on X, Y and Z axis when using the hardware joystick, the joystick control or when the following commands are used: **Go to this point**, **Go to this slide** and **Move one FOV**. After you have adjusted the speeds, click the **Done** button to save them. If you want to cancel, just press **Esc** or click elsewhere outside the popup. (To adjust the stage speed during acquisition, go to **Tools** → **Options** → **Scan Settings** → **Speed**.)

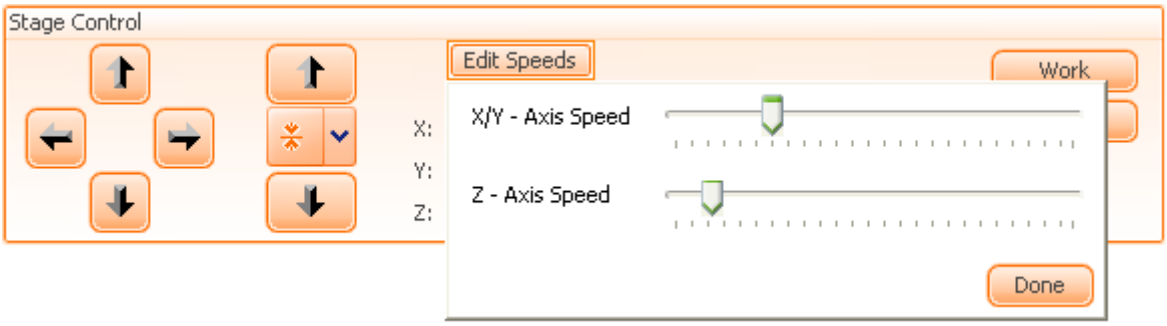


Figure 149 - Editing speeds for joystick and Z axis

- **Load** (Load): this button moves the stage down to the load position, so you can add or remove slides.
- **Work** (Work): this button moves the stage up to work position.

Note

- These stage controls work only with motorized focusing drive.
- If you are working with a **TissueFAXS i** system, which uses an inverted microscope, the objective revolver will be moved down and up rather than the stage.

5.9. Camera Control

The camera control can be used to operate the camera to display a live preview, capture images and adjust camera settings.

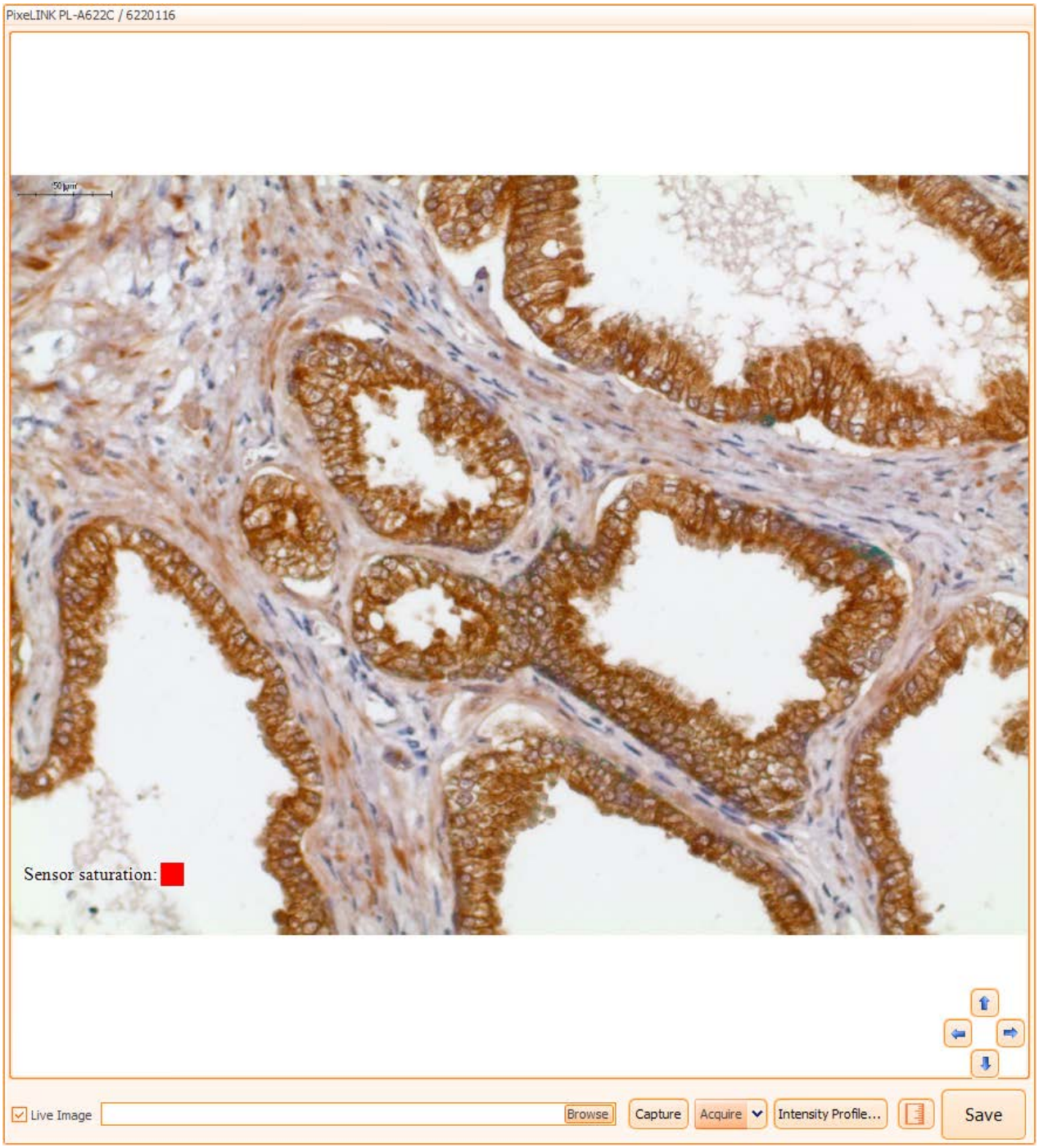


Figure 150 - Camera control

View live preview

When working with **TissueFAXS**, you may choose to see the **live image** or to disable it, by simply using a checkbox.

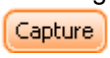
- To enable live preview check **Live Image** (Live Image);
- To disable live preview uncheck **Live Image** (Live Image).

Note

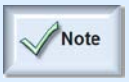
- Any time you press **View** button from the channel list, the **Live Image** will be enabled.
- If the function **Live Images** is disabled during acquisition, the overall acquisition speed will slightly increase.

Capture images (manual procedure)

Before manually capturing an image (without using the automatic acquisition feature of the **TissueFAXS** software), you must first choose a location and filename by pressing the following button:

. Next, you are able to capture the image by pressing the 

button.

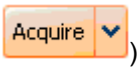


- If the filename you want to create already exists, the **TissueFAXS** software will not overwrite the existing file. The new filename will be incremented by a numerical extension that will generate a file name that does not already exist. For example, if you choose the filename *C:\image.bmp,* your new image will be automatically saved as *C:\image.bmp,* and, if the file already exists, the name will be *C:\image 001.bmp.*

Acquire Single FOV (manual procedure)

Acquiring a FOV means capturing one image (in case of multi color fluorescence - one image per reflector). **Reflectors** are to be found in the channels list in the **Acquisition** settings for the current experiment. It is necessary to have a current experiment created in order to acquire a single FOV. In addition, be sure that the camera settings for all reflectors have been properly adjusted before acquiring an image.

Figure 151 - Acquire FOV panel

Clicking on the right side of the **Acquire** button (

) will pop-up the following menu:

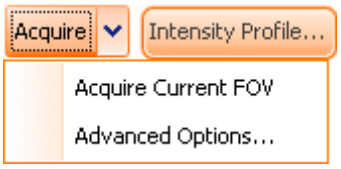
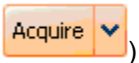


Figure 152 - Acquire FOV menu

Pressing the **Acquire** button (

) is the same as choosing **Acquire Current FOV** from the pop-up menu.

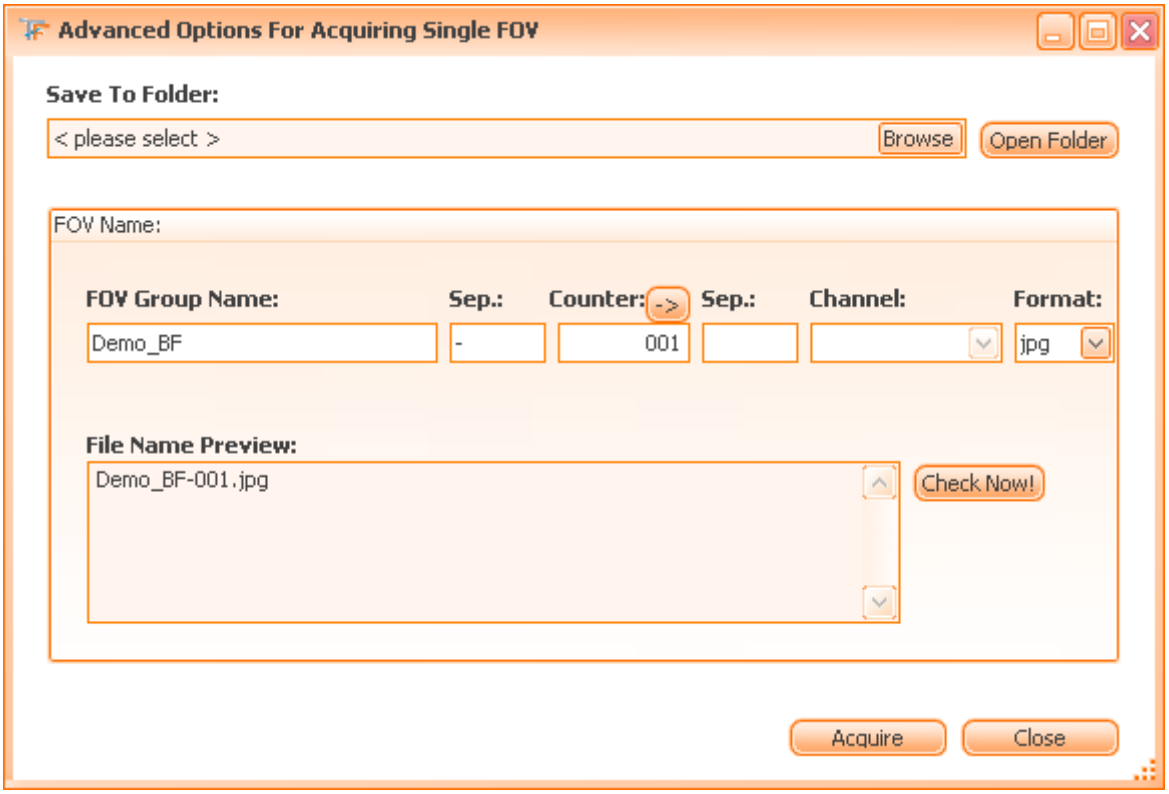


Figure 153 - Advanced Options for Acquire FOV

You also have the possibility to adjust the **Advanced Options**. The first time you attempt to acquire, you will be prompted to go to the window shown above, where you will be asked to provide the following information:

- The **storage folder** for your acquired images;
- **FOV Group Name**, which acts as a naming template for files to be acquired;
- **Separators** inside the filename (the character used as a separator should not be used as part of the filename provided by the user);
- **Counter** is used to avoid duplicate filenames when pressing the **Acquire** button multiple times;
- **Channel** (usually, it is the reflector name);
- **Format** of the graphics file.

Note

- **Filename Preview** section contains the file or the list of files obtained after the acquisition process. At any time, you can press the Check Now! button to verify the validity of the filename in the specified location (e.g. if the name is valid, if you have specified an existing folder, if you have enough rights etc.).

Intensity Profile

An **Intensity Profile** represents a set of camera related settings that you can save for further use, in order make available different combinations of settings for the acquisition process.

Figure 154 - Profile Options menu

- **Save**: the current camera settings can be saved in order to make them available for further use. You must specify a name and a short description for each profile. A default name is already generated. It contains the camera name and type, the objective magnification and the reflector selected on microscope; you can also add your own information to the already existing name.

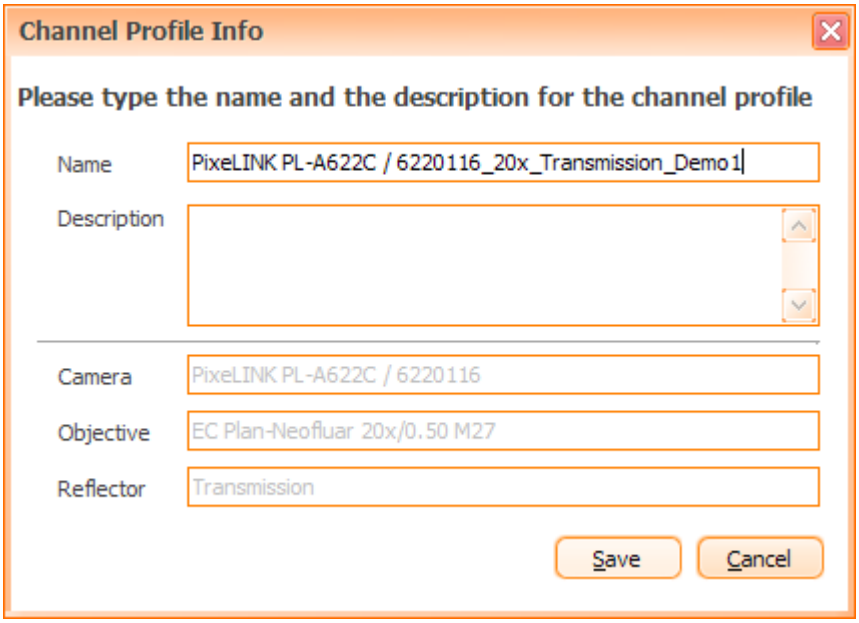


Figure 155 - Save Profile dialog

- **Load**: an existing intensity profile can be loaded for a camera. You must select any profile you want from the existing list.

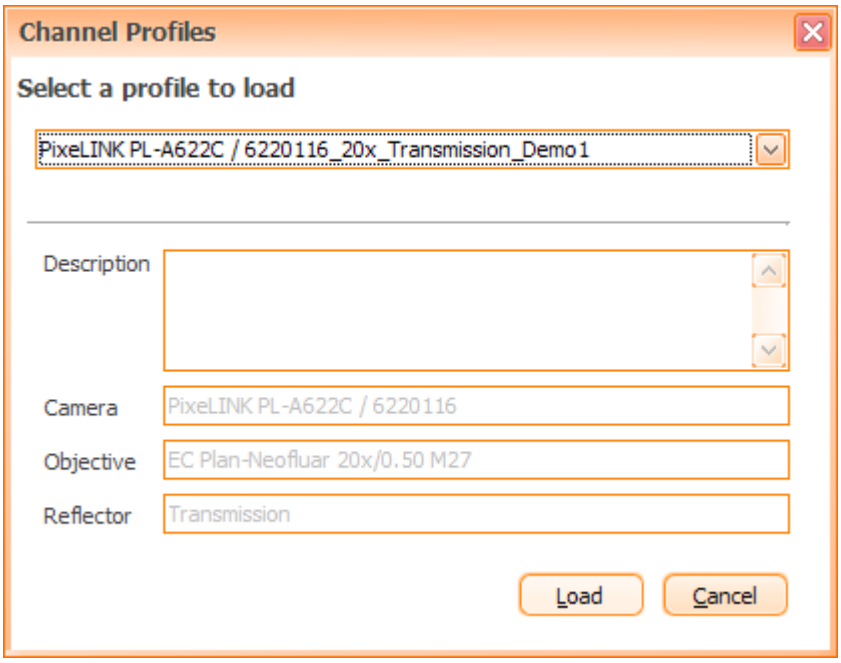


Figure 156 - Load Profile dialog

- **Delete**: if an existing intensity profile is no longer needed, it can be deleted by selecting it and then pressing the **Delete** button.

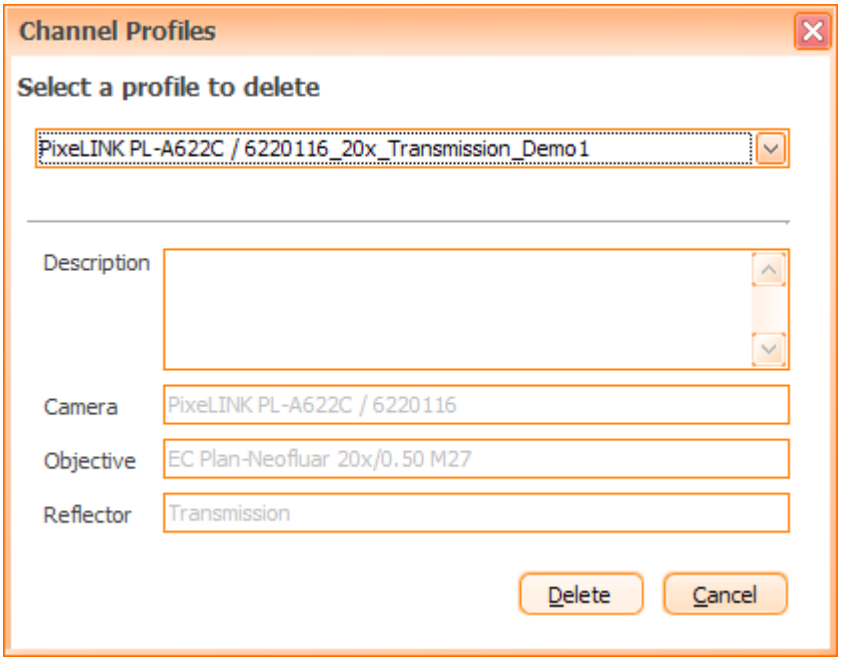


Figure 157 - Delete Profile dialog

Move by One FOV

With these buttons you can move the live image exactly one field of view up/down or left/right (only available after stage calibration).

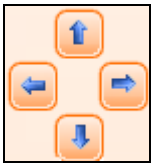


Figure 158 - Move one FOV controls

Double click to center

Double-clicking on any point in the live image will center the image at that point.

Adjust camera settings

To adjust the camera settings click on the title of the settings page.

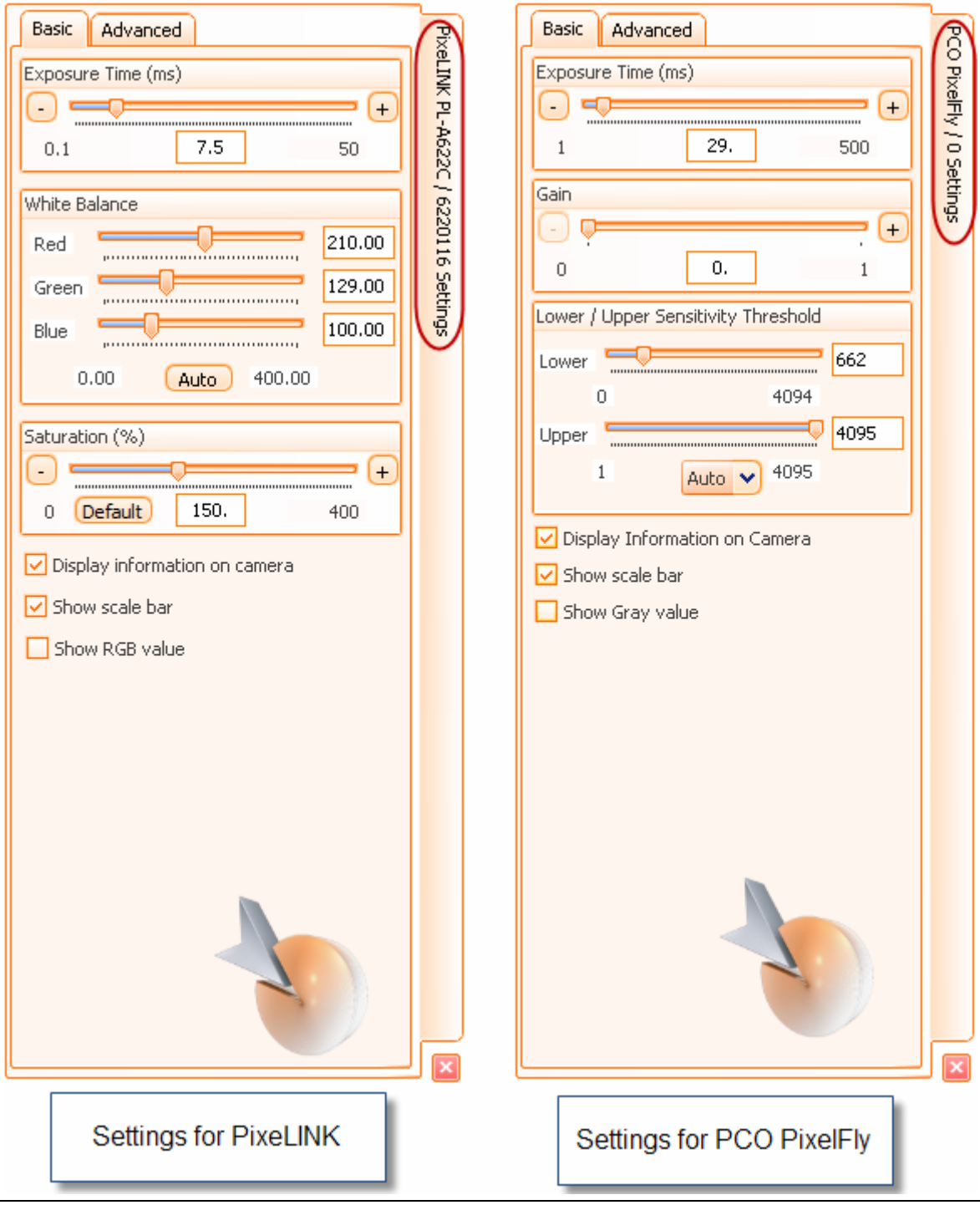


Figure 159 - Adjust camera settings tab

Press the button from the lower right corner to close the tab.

For more information please see **Chapter 5.7** of the current manual.

5.10. Drag-drop panel

In this panel, you can drag and drop controls from the microscope panel that are used most frequently. If a control is no longer needed, it can be dragged and dropped on the remove button (). When **TissueFAXS** is restarted, the last configuration is loaded.

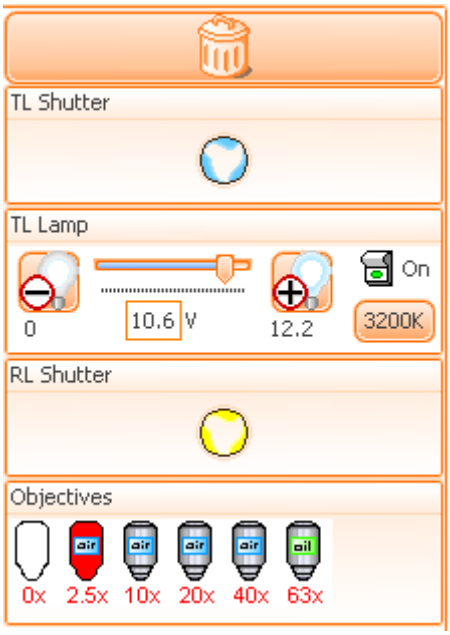


Figure 160 - Drag-drop panel

5.11. Camera settings for Preview and Acquisition

To adjust the camera settings for **Preview** or **Acquisition**, click the View button for the desired channel and its current settings will appear in the camera panel. After you adjust the camera settings, click on the Save button in the camera panel to save them.

5.12. Making a preview

You will be ready to capture a preview image of your slide when you have set up your experiment and adjusted your camera settings for your preview objective. To acquire, press the **Preview** button () located within your selected slide panel.

To preview another slide or multiple slides, click on the drop-down menu of the **Preview** button for more preview options.

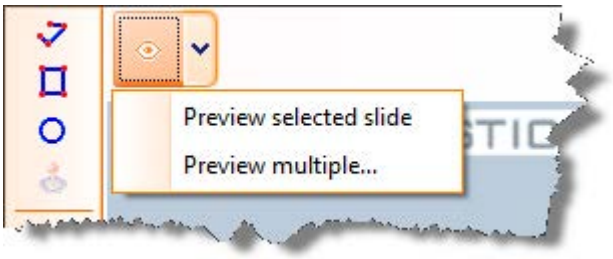


Figure 161 - Preview options panel

If **Preview Multiple** is chosen, the following dialog will appear:

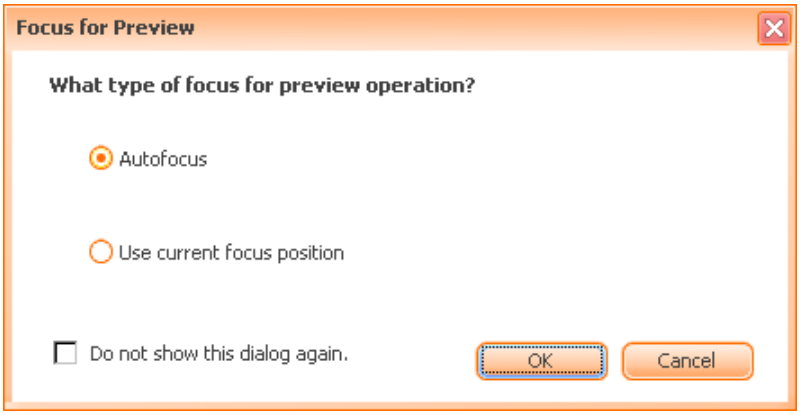


Figure 162 - Focus for Preview dialog

Select your focus method then press **OK**. In the next window, select the slides you wish to acquire.

- Autofocus method will use the method defined in **Options → Scan Settings → Focus**. If you check **Do not show this dialog again,** your current choice will be remembered for future use and you will not be prompted again. This selection can be reset from **Tools → Options → Application Options → Remember**.

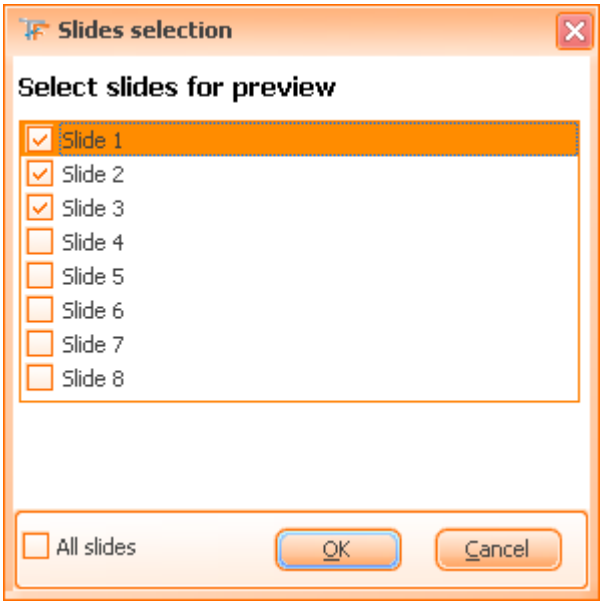


Figure 163 - Slides selection checkbox

5.13. Stopping a Preview

To quit the preview, there are **two options**: you can abort the preview of the current slide in progress or the complete preview process.

To quit the preview of the current slide (current selection), simply press the **Preview** button ( ), which has turned into a power-off-button during acquisition. **TissueFAXS** will quit the current slide and move on to the next slide, if available.

To quit the complete process, click the **Preview Selected Slide** button ( ) from the very top panel.

5.14. Experiment Editor

The **Experiment Editor** is the drop down menu located at the left side of the application's main window. Its purpose is to organize your slides, regions of interest and acquired images into one complete list. Regions of interests are displayed as thumbnails images in this list (see the image below).

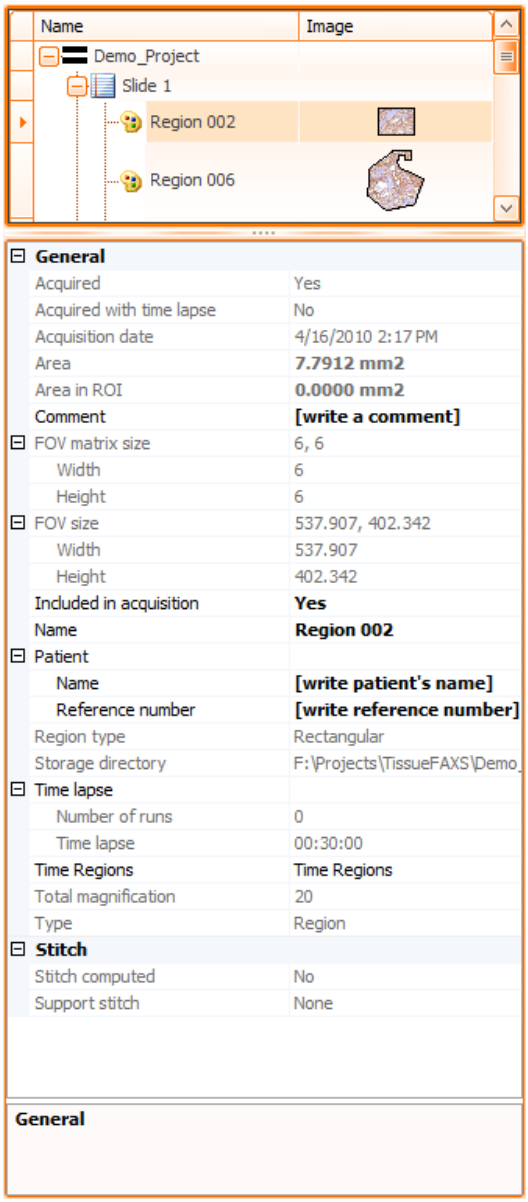


Figure 164 - Experiment editor: Experiment with slides and regions

From top to bottom, the editor consists of **three main parts**: a **tree-like representation of the experiment,** a **property grid,** and a **description area**.

Experiment tree-like representation

In **TissueFAXS**, you can draw a region on your preview image (with the rectangle, circle, or custom draw tool), as described in **Chapter 5.16.1**. The **Experiment Editor** will automatically display each new region in the tree list along with its corresponding thumbnail. This gives the user a visual overview of all regions of interest in the experiment (that can be acquired later).

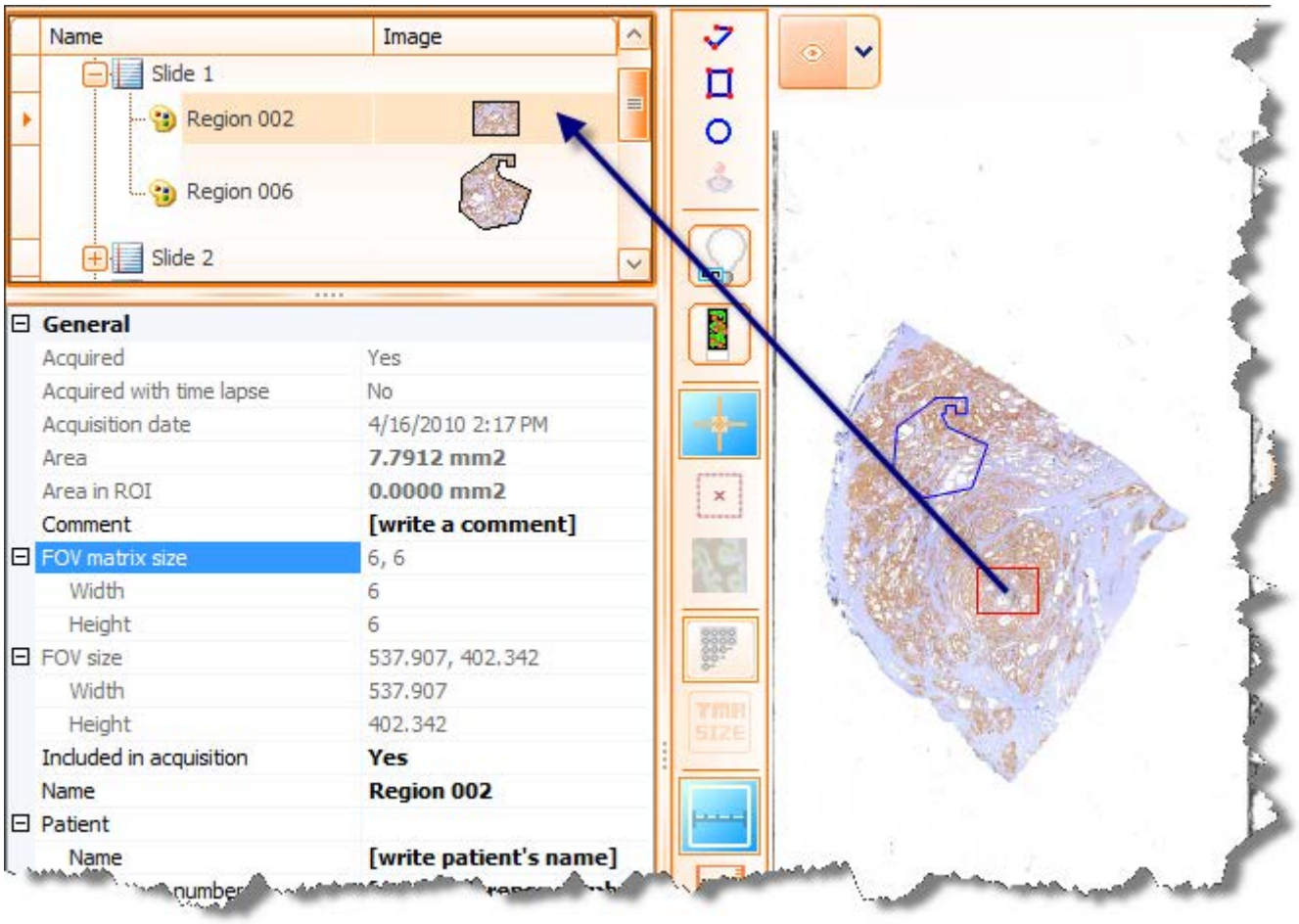


Figure 165 - Thumbnail for regions

Grouping Regions

This is one important feature of the **Experiment Editor**, allowing regions to be grouped together. This is particularly useful when you wish to distinguish multiple regions of interest that exist on the same slide. For example, you may have two patients' tissue samples on the same slide and want to organize your regions/images into a group for each patient. To **create a group**, right-click on the slide in the list and select **Create Group**. Once created, the group can be renamed and regions can be dragged and dropped into the respective group.

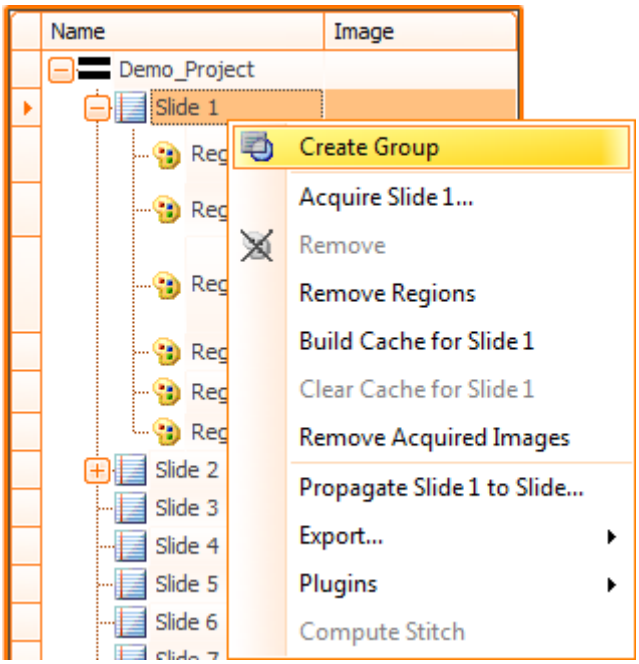


Figure 166 - Creating groups context menu

Right-clicking on items in the menu allows you to directly acquire or remove them from the experiment.

Acquire experiment item

Clicking on this option allows you to directly acquire the selected experiment item.

Remove

Clicking on this option allows you to directly remove the selected item from the experiment.

Remove Regions

Clicking on this option allows you to directly remove all items from the selected slide/group from the experiment.

Build/Clear Cache for Slide

Builds/clears cache for the selected slide.

Remove Acquired Images

This option will erase all the acquired images and associated files from the storage area. Notice the fact that this option is available only for acquired regions.

Propagate region/slide to slide…

This option copies selected regions from a slide to other slide/slides that you can select in the dialog below:

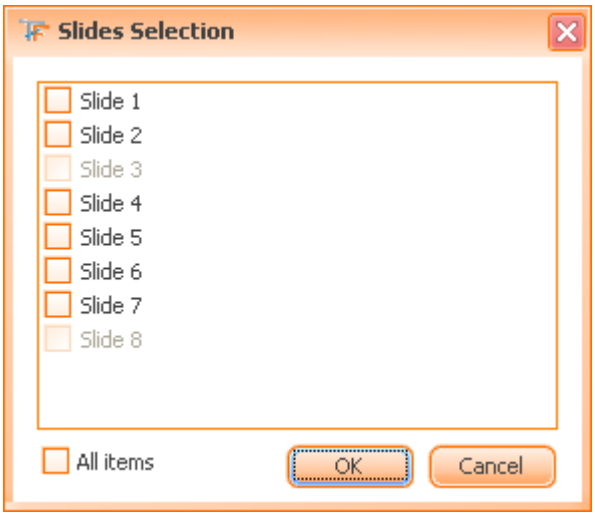


Figure 167 - Slide selection for propagation checkbox

TIP

- You can select any slide to propagate regions, except for the slides that appear disabled in the **Slide Selection** dialog.
- A slide appears disabled because:
  - It is the slide containing the original regions to be propagated afterwards (you cannot propagate a slide to itself);
  - The content type is different from the type of the slide containing the original regions to be propagated.

The dialog contains another option (**All items**) that will select all available slides.

Note

- The propagated regions will not have corresponding thumbnail images.

Export…

To find out more about this option, please see **Chapter 5.20** of the current manual.

Note

- For certain operations (acquisition, propagation, export) you have the possibility to use the multiple selection.

Plugins (see **Chapter 5.21**)

Compute Stitch (see **Chapter 4.3.2**)

Property Editor

The **properties** section is located below the tree-list. More precisely, it is a property grid that shows all the properties available for a specific project item.

A project item can be a slide, a region, a group and the experiment itself. Some of the properties displayed in the properties grid are editable, others are read-only. Here, you may change/create names for slides, groups, and regions. The user can also choose to include/exclude a region in the acquisition process or determine if the region is already acquired.

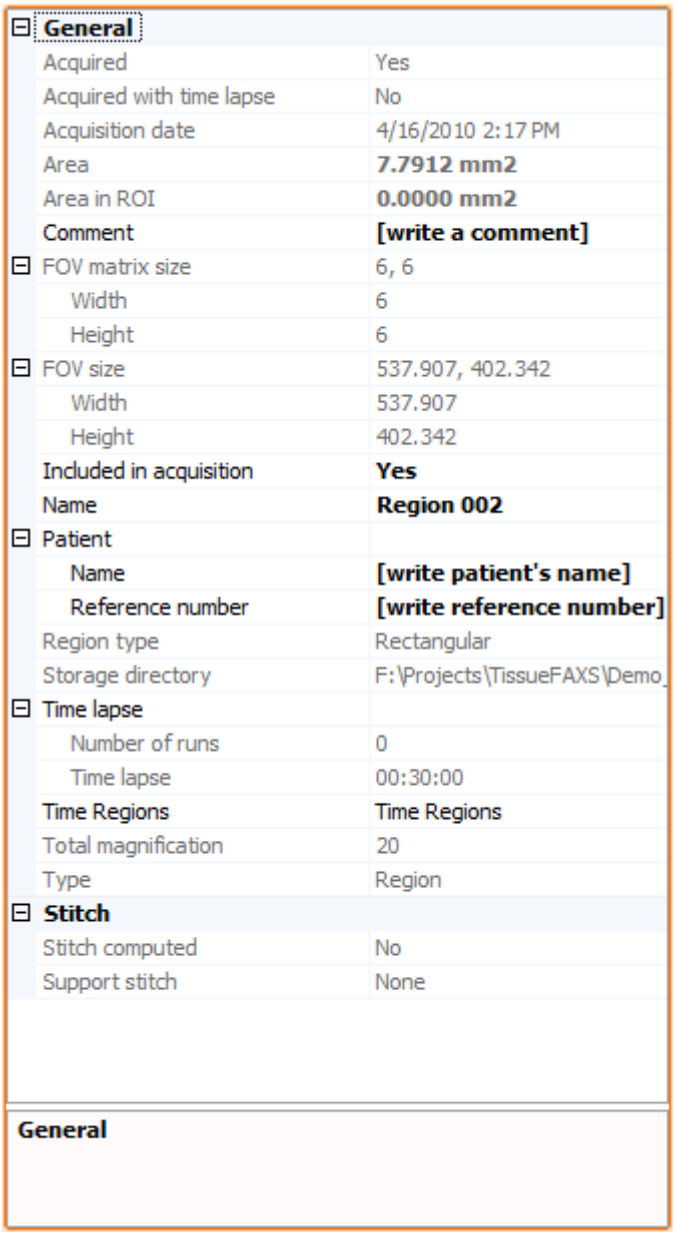
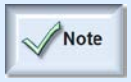


Figure 168 - Region properties section

In the example above, the region has the following properties:

- The item is acquired;
- The item is not acquired with time lapse;
- Area: total area of the region (including only acquired fields of view);
- Area in ROI: area for the cropped shape (including only acquired fields of view);
- The acquisition date;
- The comment indicates sample related data;
- The item consists of a virtual matrix containing 6x6 Fields of View;
- The FOV size (Width and Height);
- The item is included in acquisition;
- The item name;
- Data concerning the patient;

- The type/shape of the region is rectangular;
- The storage directory is shown here;
- Time lapse: number of runs and time lapse are shown here;
- The total magnification;
- The type of selected item is *Region*;
- Stitch.



- By selecting more items at the same time (*multiple selection*), all the common properties shared by these items can be simultaneously modified in order to have the same value.

- Some of the items from the Property Editor are not editable (they appear in grey).

- The **properties** of an experiment can be displayed by going to **File → Properties....** In the dialog that appears, only the **Comments** concerning the experiment can be edited.

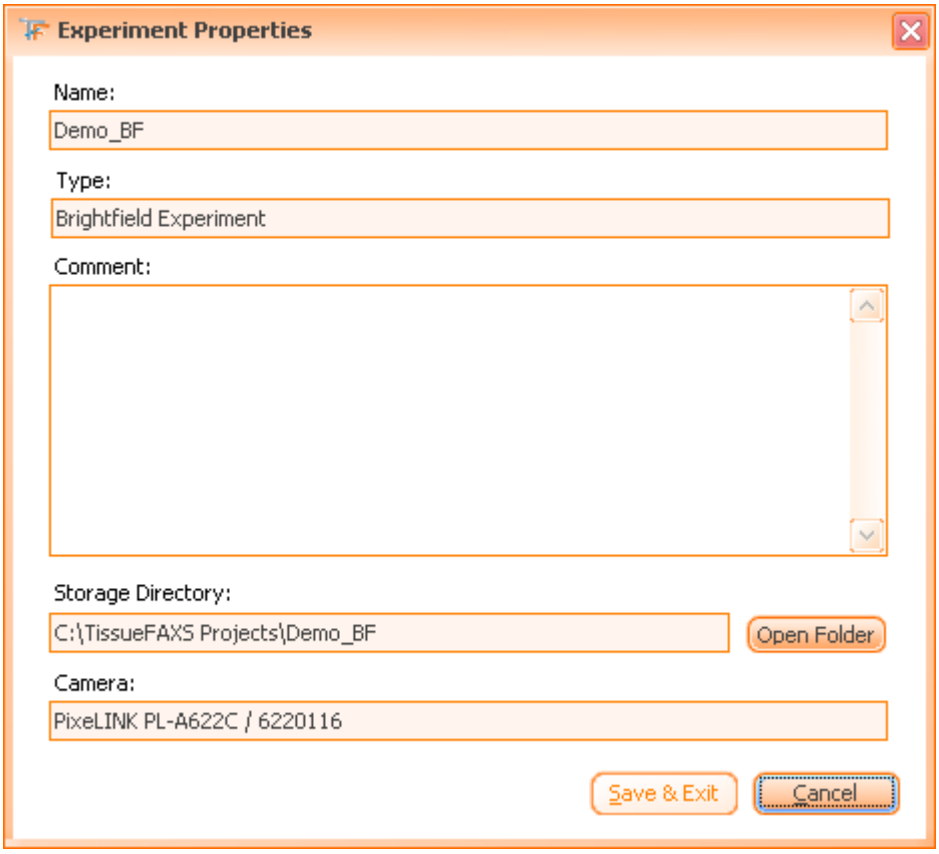


Figure 169 - Experiment Properties dialog

5.15. Slide Manager

The **Slide Manager** is a feature that allows a quick and simple access to the main slide settings.

It can be accessed from:

- **File → Slide Manager**

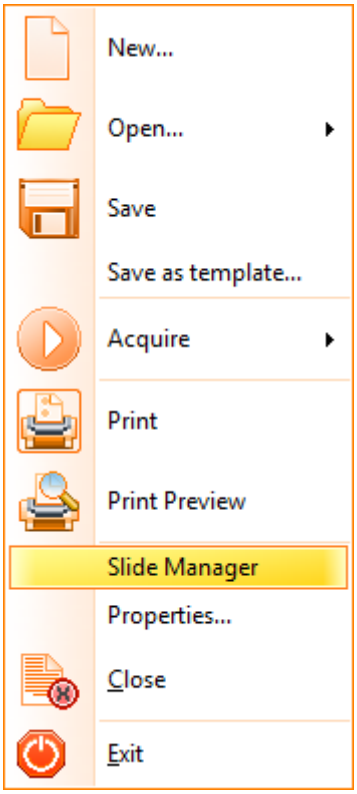


Figure 170 - File Menu: Slide Manager option

- **Experiment Editor**: right click on the name of the experiment and choose **Slide Manager** option from the contextual menu.

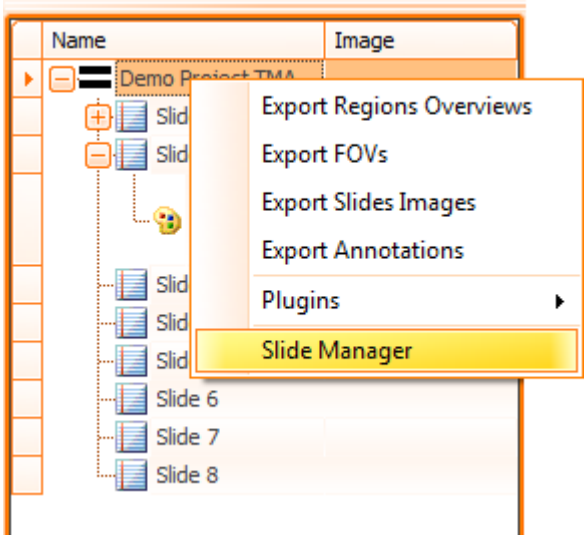


Figure 171 - Experiment Editor: Slide Manager option

In both cases the **Slide Manager** dialog will open:

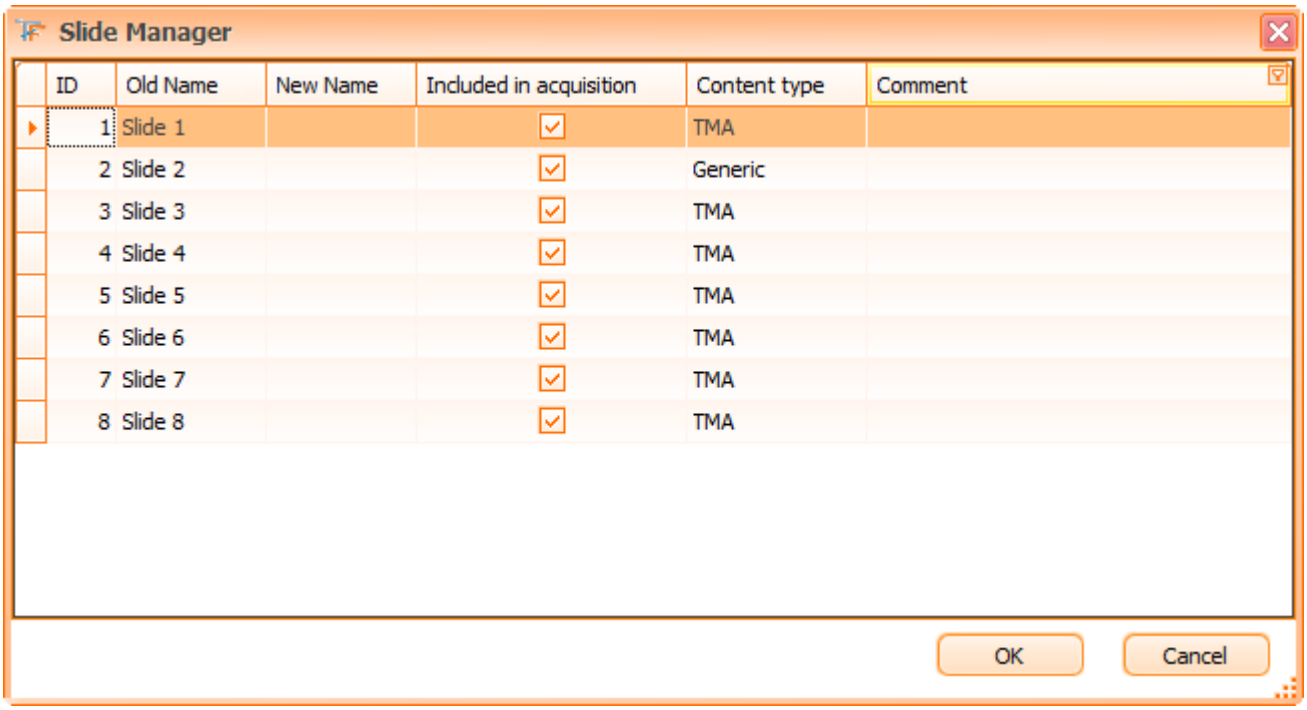


Figure 172 - Slide Manager dialog

Here you can perform the following actions:

- Change the name of the slide: type the new name into the **New Name** field of the desired slide;
- Include the slide in acquisition by checking the corresponding checkbox in the **Included in acquisition** column;
- The **Content type** column informs you about the type of each slide;
- You can also add a comment to each slide by typing it into the **Comment** field of the desired slide.

5.16. Slide Editor

The slide editor is one of the main features of the **TissueFAXS** application. It displays the image of the whole slide and also a panel with all the applicable functionalities.

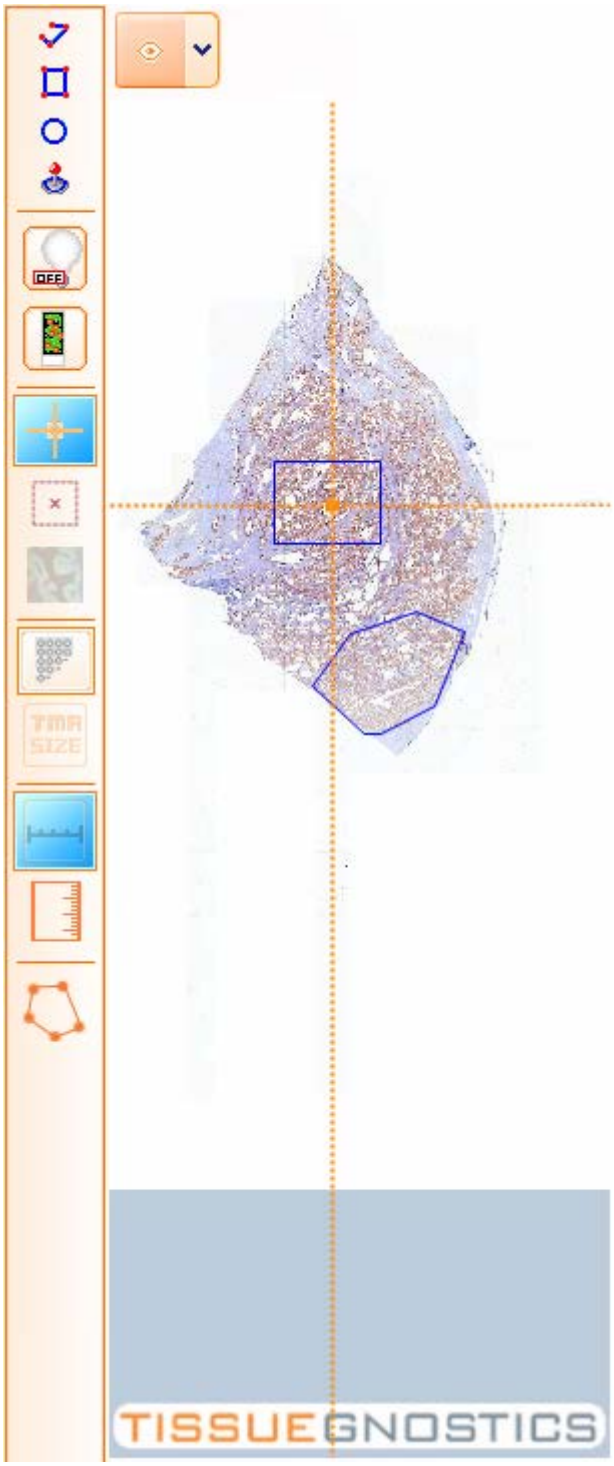


Figure 173 - Slide Editor in the main window

A slide preview must be acquired to use the slide editor. You can perform a set of operations on a slide by right-clicking on it and generating a pop-up menu, as shown below:

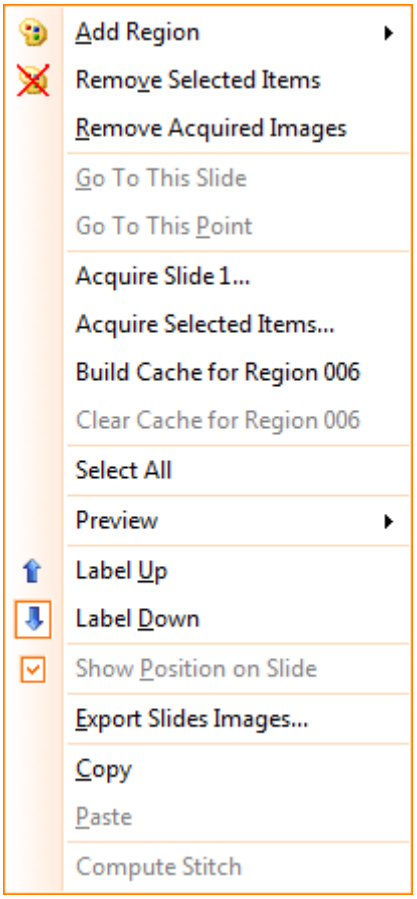


Figure 174 - Slide editor context menu

5.16.1 Add Region

In this section you will learn how to draw regions on your slide. This option yields a submenu which contains four types of drawing tools.

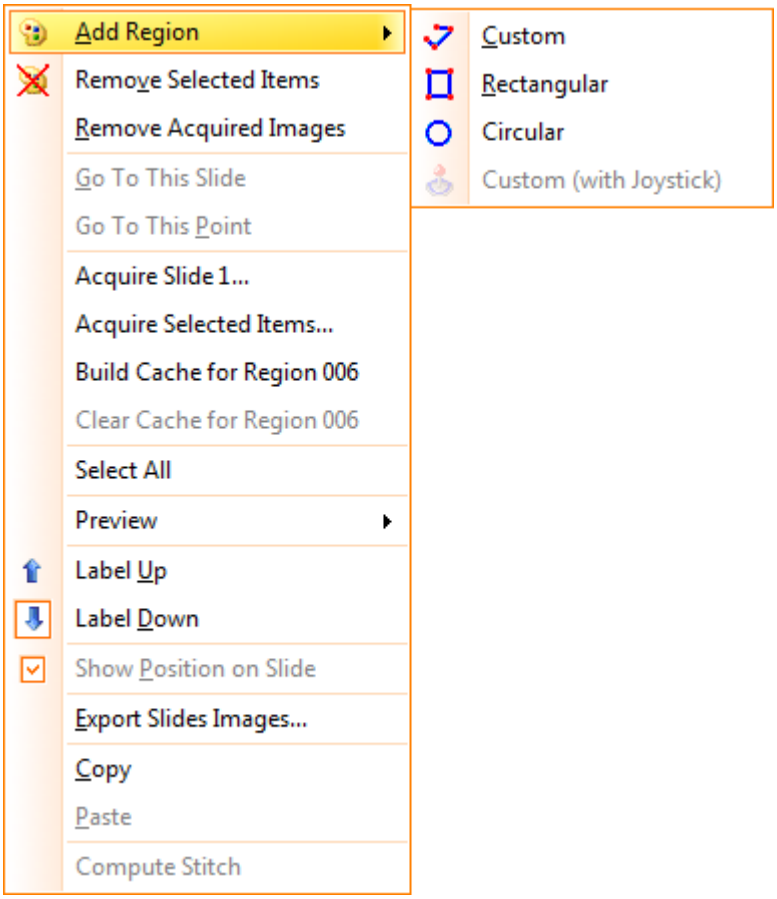


Figure 175 - Add region context menu

There are four different **methods** for drawing your regions of interest:

- **Custom** - This method allows the user to create a custom shaped region with a rubber-line tool. The tips of the line are made static by left clicking on the image. Simply right click anywhere to close your region.

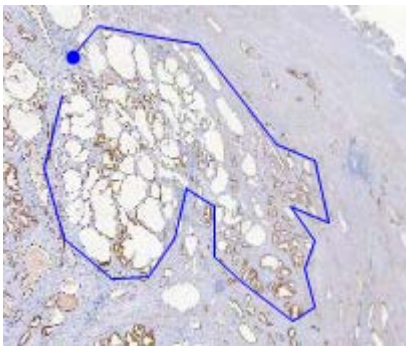


Figure 176 - Creating custom region

- **Rectangular** - This method allows the user to simply drag a box over the image to create a rectangular region. A start point and an end point are chosen by left clicking on the image.

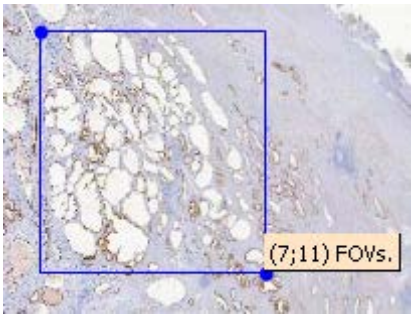


Figure 177 - Creating rectangular region

- **Circular** - This method requires two left clicks to create a circular region. The first left click determines the center while the second click determines the radius of the circle.

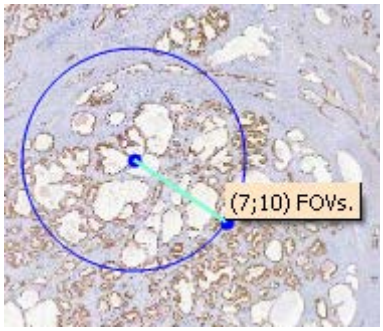


Figure 178 - Creating circular region

- **Custom (with joystick)** - This method allows you to draw the region shape by using the hardware joystick. When you select this, the following dialog will appear:

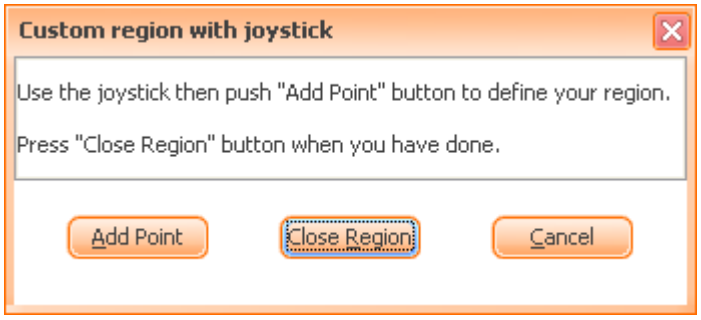


Figure 179 - Custom region with joystick dialog

Move the joystick to the desired points and for each of them click the **Add Point** button. When you wish to close the region, click the **Close Region** button. Usually, this function is used in conjunction with **Live image** (using a high magnification in order to increase the precision of the region shape). You can click anytime the **Cancel** button to reject any changes you have made.

5.16.2 Modify Region

All created regions can be modified (resized, moved) as long as no images have been acquired for them.

- The **move whole region** operation can be performed in 2 steps:

1. Select the region by right clicking an inner point of its shape;
2. Click and hold the right mouse button in the same spot to begin the drag and drop operation.

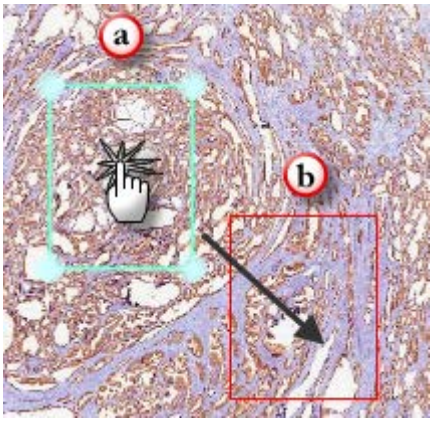


Figure 180 - a) Click an inner point of the region

b) Drag&Drop operation to move the region

- The **resize** or **reshape** operation:

Each region has special points that can be dragged in order to resize it or to change its shape, depending on the region type, as described below:

- For all the regions, these points can be seen when the mouse hovers above the region.

For **Custom Regions**, only the currently selected point can be moved (it appears marked by a little black cross, like in the image below), so you have to modify all desired points one by one in order to reshape the region.

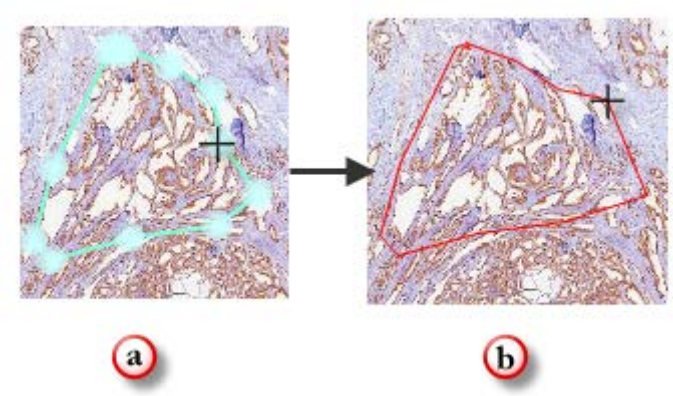


Figure 181 - a) Resize (move point) custom region

b) Drag&Drop operation to move the point

For **Rectangular Regions**, the entire rectangle is resized when you move the selected point (it appears marked by a little black cross, like in the image below).

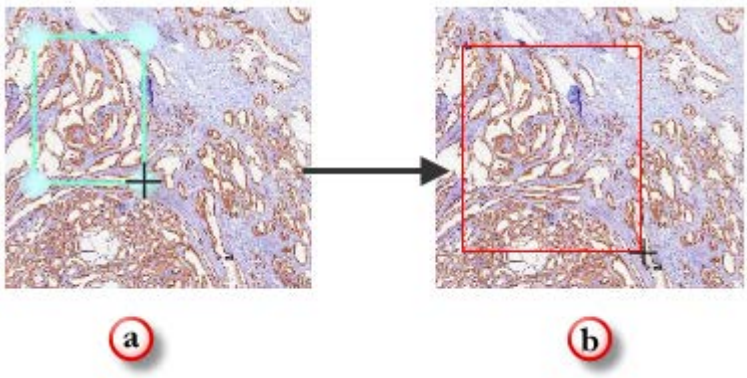


Figure 182 - a) Resize (move point) rectangular region

b) Drag&Drop operation to resize the rectangle

Likewise, **Circular Regions** are resized by moving the selected point (it appears marked by a little black cross, like in the image below).

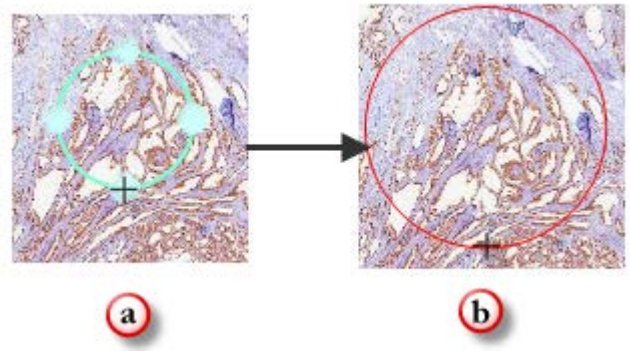


Figure 183 - a) Resize (move point) circular region

b) Drag&Drop operation to resize the circle

- **Remove Region**: right-click on the region and choose **Remove Region**.
- **Remove acquired images**: this option will erase all the acquired images and associated files from the storage area. Notice the fact that this option is available only for acquired regions.

5.16.3 Copy and Paste region

- **Copy Region**: this operation can be done by choosing the **Copy** option from the region contextual menu or by using the **Ctrl+C** combination from the keyboard.
- **Paste Region**: this operation can be done by choosing the **Paste** option from the region contextual menu or by using the **Ctrl+V** combination from the keyboard.

- It is possible to select multiple regions to be copied, by using the mouse selection.

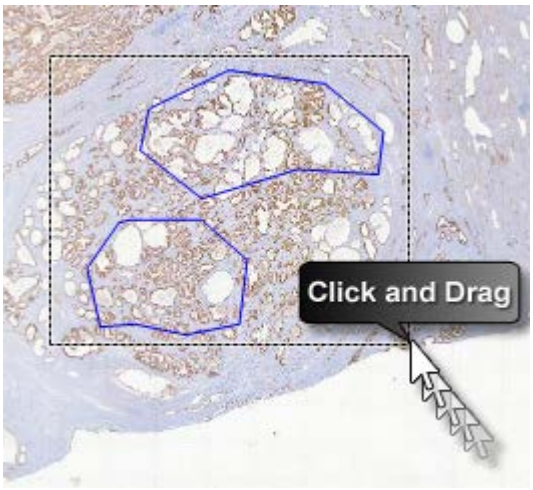


Figure 184 - Multiple selection of regions to be copied

5.16.4 Preview Area

A region can be set as a preview area for future preview operations in the current experiment.

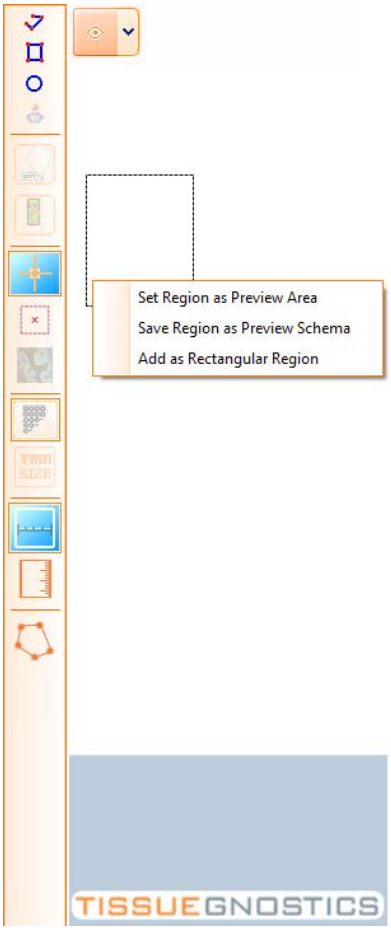


Figure 185 - Set new preview area context menu

If the preview area chosen in the experiment setup needs to be adjusted, a rectangle can be drawn on the slide, clicking the left mouse button to accept a drawn rectangle. Right-clicking within the selection will allow doing the following:

- **Set region as preview area** - set current region as preview area for current experiment. This will affect all subsequent slides in this experiment.
- **Save region as preview schema** - saves current region as preview area for future use and set it as preview area for current experiment. You can give a name to this preview scheme and store it (to use it again in future experiments).
- **Add as rectangle region** - creates a region that will be acquired later.

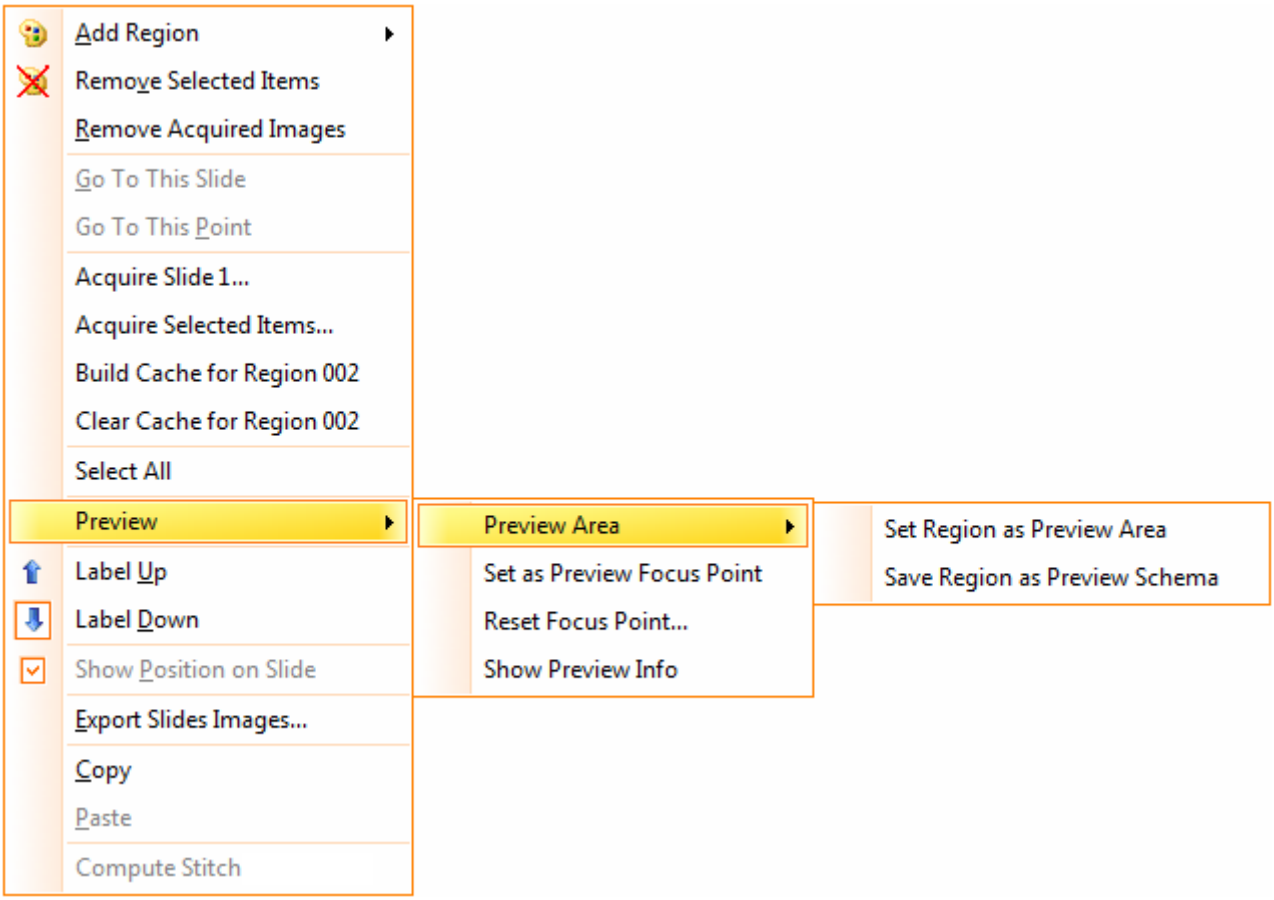


Figure 186 - Preview Area context menus

5.16.5 Stage Menu

By right clicking on any of the slide images, you will obtain a contextual menu including more options, as seen in the image below:

Figure 187 - Stage Control: context menu

This contextual menu will help you better manage the physical stage and control the acquisition process.

- **Go To This Slide** -To make the objective lens physically move onto a particular slide, you must right-click on the slide and choose **Go to This Slide**. In the main window, a green box will outline the slide that is currently in observation position.
- **Go To This Point** - To make the objective lens physically move to a specific location on a slide, right-click on that point and choose **Go to This Slide**. This is particularly useful for navigating through acquired slide previews and for determining where to draw regions of interest in the Custom (with Joystick) mode.
- **Acquire Slide -** this item starts the acquisition process. Its target is the selected slide only.
- **Acquire Region** - starts the acquisition process for the selected region only.
- **Label Up / Label Down -** The **Label Up** and **Label Down** entries allow you to specify the position of the slide on the stage. Usually, all the slides are inserted in the same way, but if this is not the case, the label position can be customized for individual slides on the stage.
- **Show position on slide** ( ) - You have the option of viewing the objective's position on the slide in relation to your preview, by selecting **Show position on slide** in the menu. An orange box appears indicating the size of one field of view along with cross interrupted lines that mark the center. The FOV size is determined by the objective in use. If the FOV is not calibrated for the selected objective, the box will have the default size.

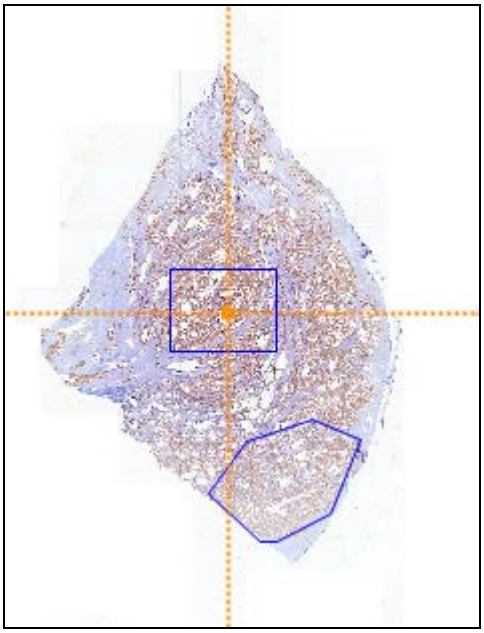


Figure 188 - Show position on slide

- **Show preview info** ( ) - By pressing this button, you will be able to see the location of the preview area (common for all slides) on the slide and the position of the focus point (for each individual slide) for preview operation. You can also access this option from the contextual menu that appears when right clicking in the slide editor.
- **Set as preview focus point** - Use this option to set the focus point to the desired position, by right clicking the respective point.

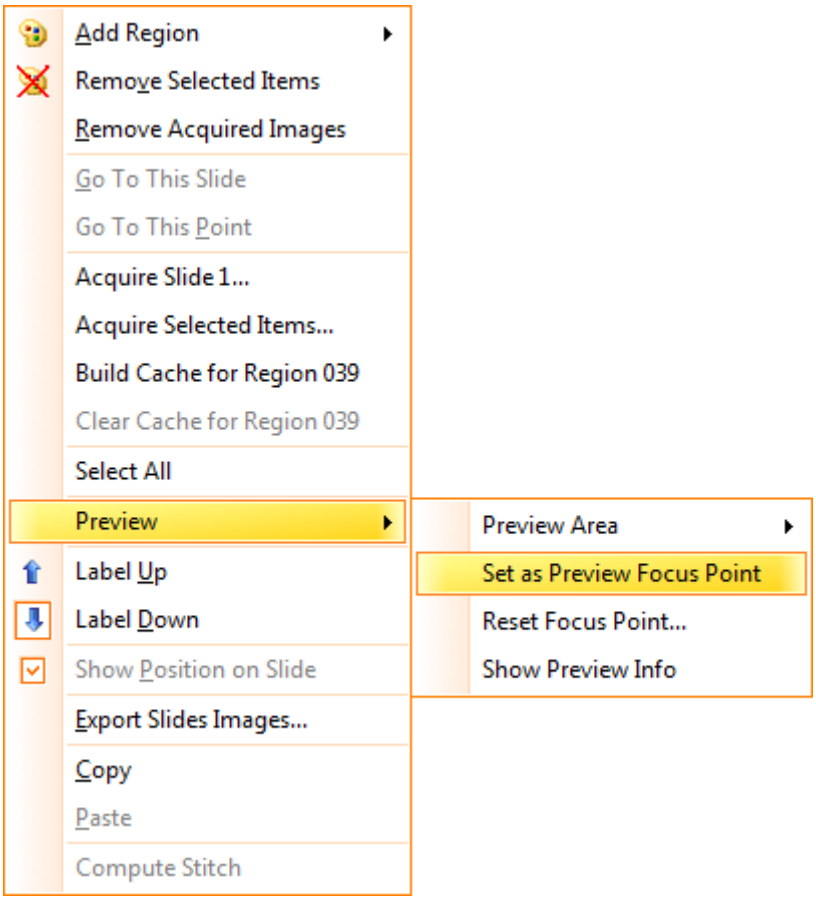


Figure 189 - Set as preview focus point context menu

- **Reset focus point…** - use this option to reset the focus point to the default position (center of preview area).

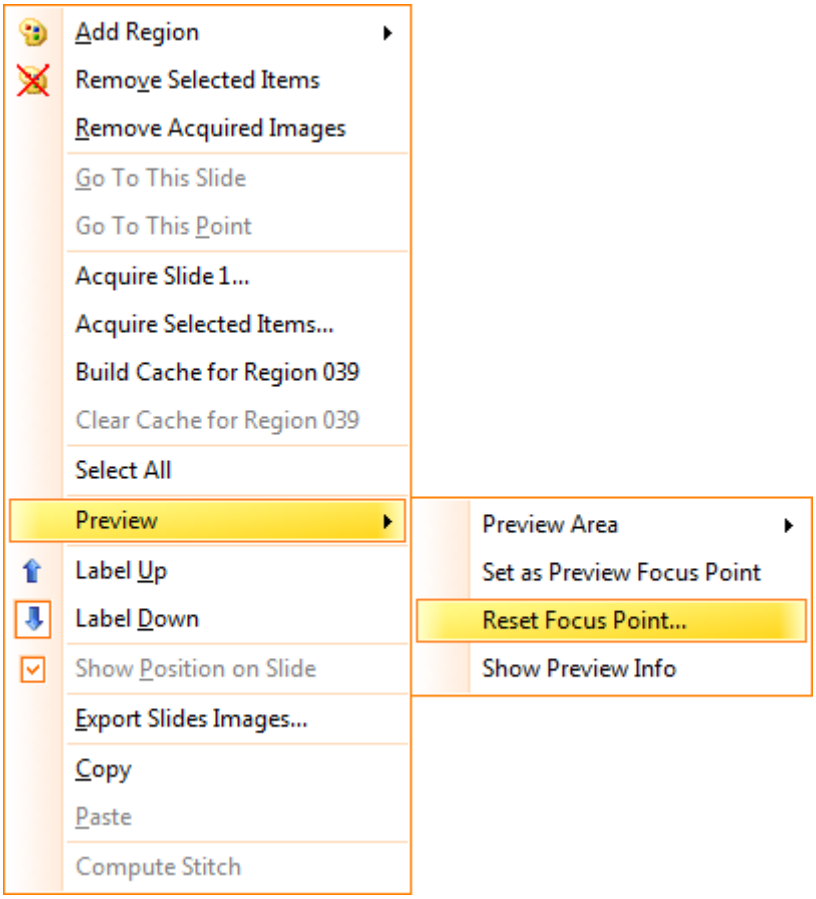
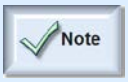


Figure 190 - Reset focus point context menu



- You can reset the focus point for the **current slide** or for **all slides**.

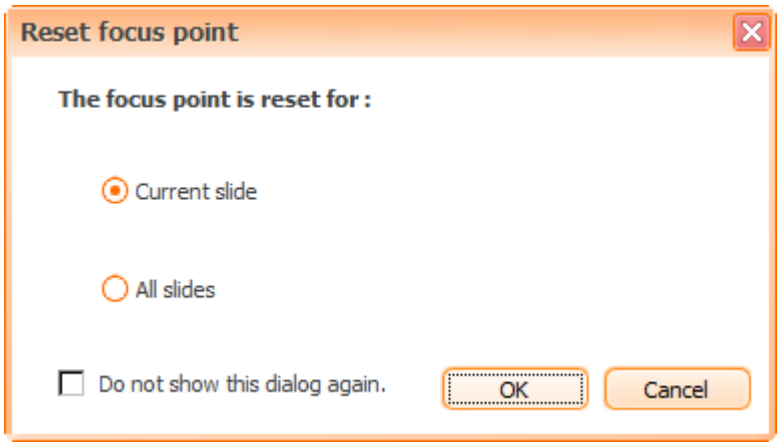


Figure 191 - Reset focus point dialog

5.16.6 Export this slide image…

To export a slide's preview image, select **Export this slide image** from the menu. You will be prompted to choose the destination directory and the encoding type for the image. For more details concerning this feature, please see **Chapter 5.20.4** of the current manual.

5.16.7 Automatic tissue detection

After the preview is done, **TissueFAXS** is able to auto-detect the tissue regions on the slide. This is a great tool provided by **TissueFAXS** that can help you save time and improve the accuracy of the tissue detection.

In order to detect generic tissue samples, please make sure that the **Content Type** property of the slide is set to **Generic**. Then press **Detect Tissue** button ( ) from the slide editor toolbar. The following dialog will appear:

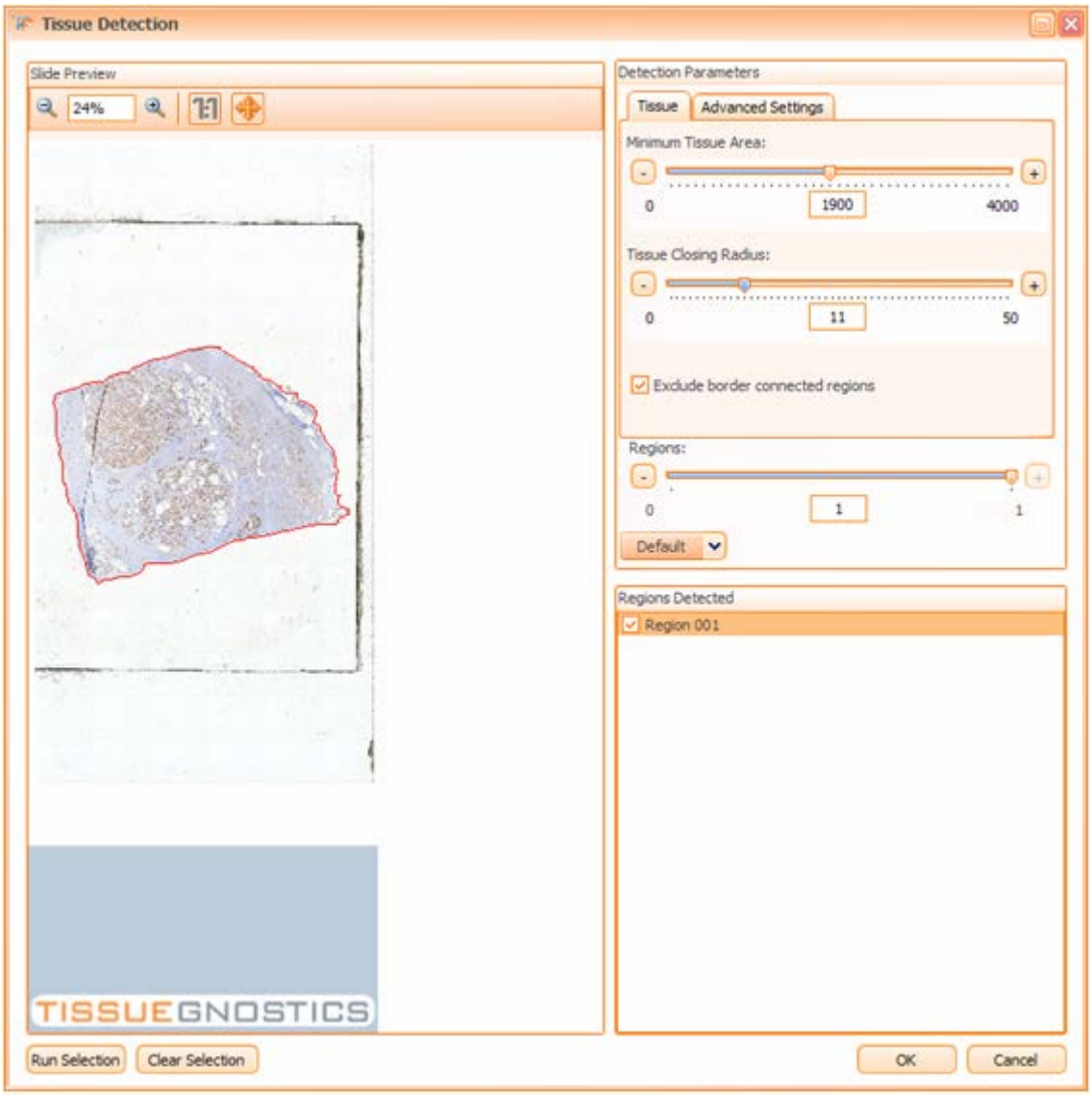


Figure 192 - Tissue Detection panel

By default, the detection is run on the entire preview image. If you want to refine the results, you can run the detection on a smaller area:

- select the desired area by drawing it on the displayed image;
- click on **Run Selection**.

If you want to cancel selection, press the **Clear Selection** button.

Detection Parameters

In order to refine the results of the automatic detection, **TissueFAXS** provides a set of parameters you can adjust according to your needs, in order to obtain the desired results.

- **Minimum Tissue Area**: the smallest area a tissue region can have. All the regions with a smaller area will not be taken into account.
- **Tissue Closing Radius:** try to increase this parameter if your region is split into multiple small subregions. The result will be a single region containing smaller subregions.
- **Exclude border connected regions:** the regions touching the border will be ignored.
- **Regions**: number of regions expected to be detected.
- **Default:** this dropdown button has two options:

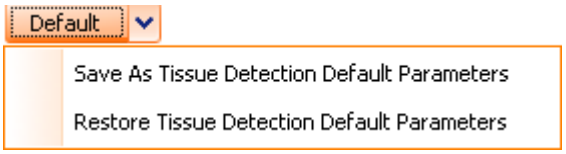


Figure 193 - Tissue Detection: Default Parameters

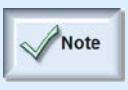
- **Save as Tissue Detection Default Parameters**: it allows you to store this set of parameters values for future detection;
- **Restore Tissue Detection Default Parameters**: it restores all the parameters to the last saved default parameters.

Advanced Settings

- **Thresholds**
  - **Automatic**: if this is checked, the **RGB** (red/green/blue) channels values are automatically determined within three *intervals* given by the user. These intervals are established by increasing the lower (default value is 5) and upper (default value is 255) values.

  If **Automatic** option is unchecked, the tissue detection algorithm will use three *values* given by the user for Red, Green and Blue channels**.**

  - **Apply same value for all channels**: by checking this option, all channels will have identical values or intervals (you must set the value or interval for the **red** parameter, this value or interval will appear automatically for the other two channels);
  - **Red**: the amount of red in the picture;
  - **Green**: the amount of green in the picture;
  - **Blue:** the amount of blue in the picture.



- Depending on the color used in the staining, different adjustments of the Red, Green and Blue values might become necessary.

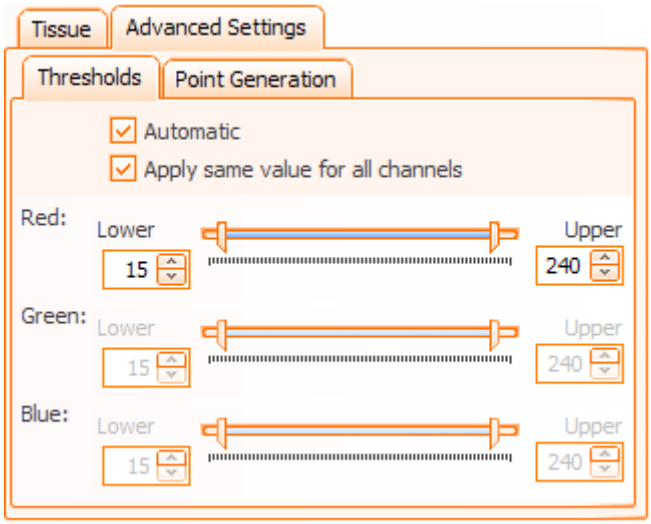


Figure 194 - Advanced Settings: Thresholds tab - Automatic checked

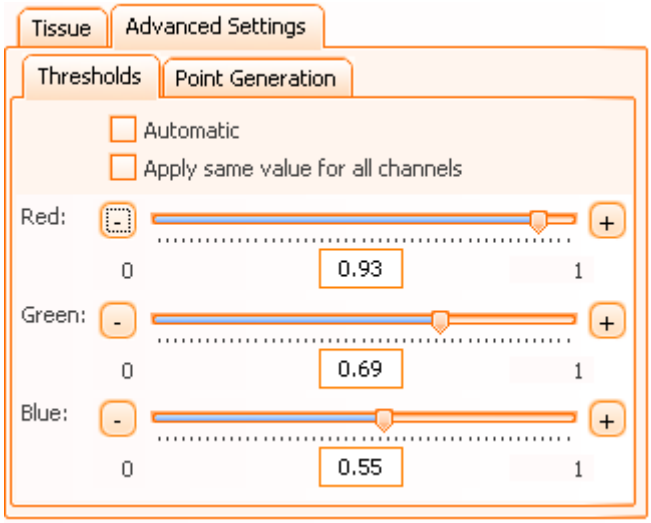


Figure 195 - Advanced Settings: Thresholds tab - Automatic unchecked

- **Point Generation**
  - **Distance**: the initially detected region will be inflated with the specified number of pixels, in order to entirely include the edges of the tissue and a small surrounding area;
  - **Max Step**: this parameter represents the maximum distance between two generated points. The smaller the distance is, the more accurately the contour of the region will be represented.

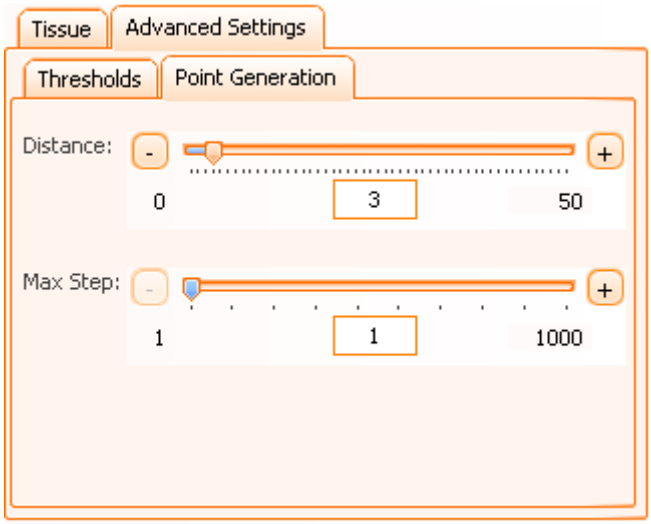


Figure 196 - Advanced Setting: Point Generation tab

For more accurate tissue detection the following controls are available:

- **Zoom in** and **Zoom out** buttons allow zooming the image. Another option would be to type the desired zoom value in the zoom editor.
- **View Original Size**: displays original size of the image.
- **View Best Fit**: displays the entire region.

5.16.8 Slide Overlay

Located in the toolbar, the **Slide Overlay** button ( ) will allow you to view your preview image for each reflector/channel (in **fluorescence** experiments). Check the desired reflector in the panel shown below in order to obtain the desired preview.

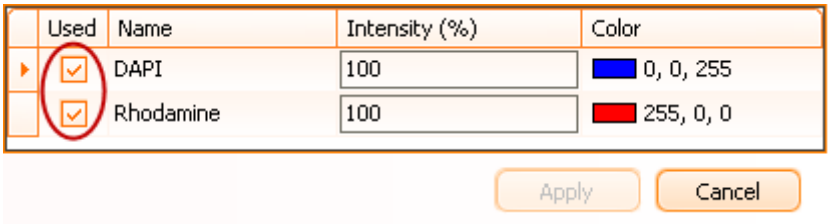


Figure 197 - Slide Overlay Dialog

5.16.9 Custom Selection

Custom Selection button ( ) allows the user to freely draw a selection over the sample in order to select the desired group of ROIs. This feature helps avoiding multiple clicks for one-by-one selection of the desired items.

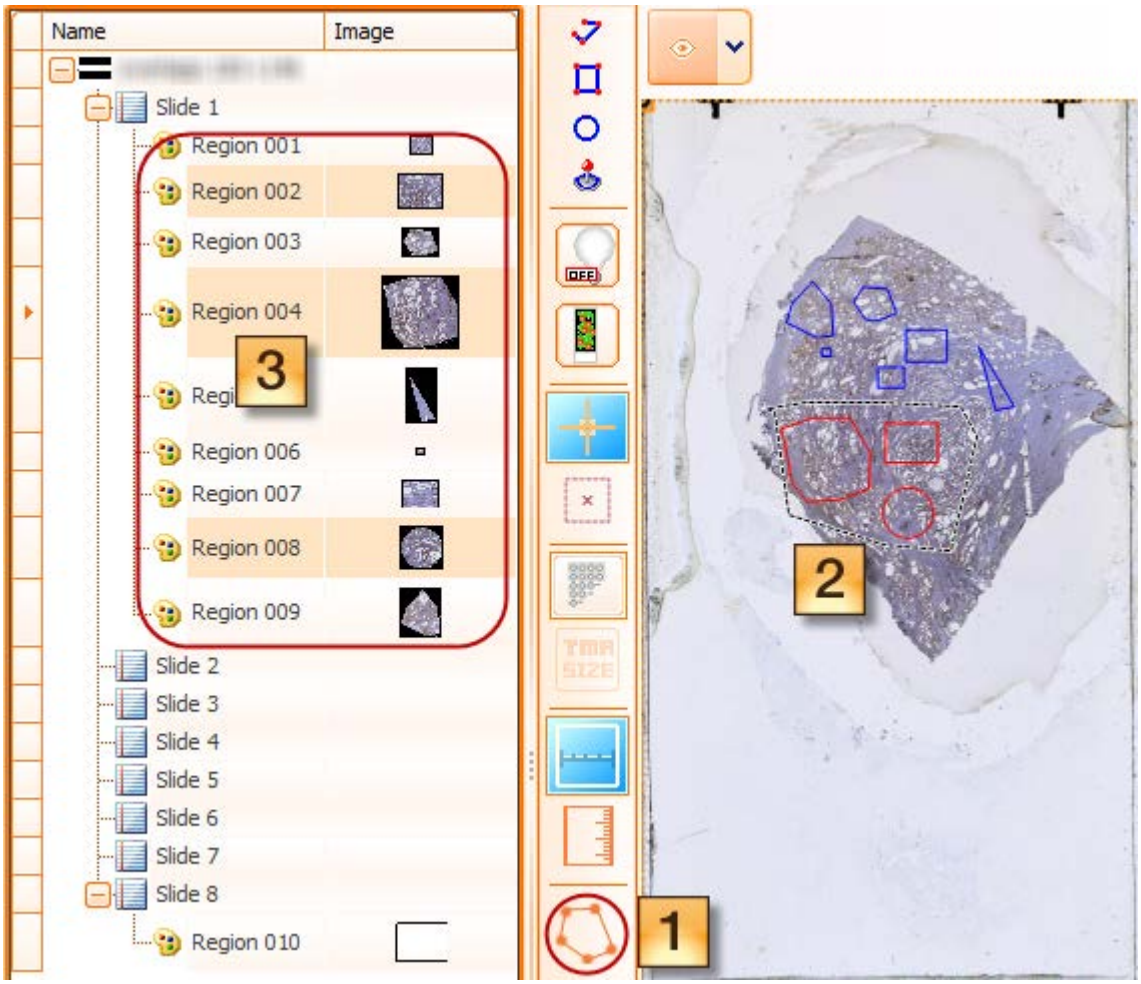


Figure 198 - Custom Selection

5.17. Region Viewer

A region is an array of FOVs disposed in a matrix structure. Each FOV has its own position within the matrix, which represents the number of rows and columns. The FOV Matrix Size property of the region represents the number of rows and columns.

To view acquired regions, double-click on the region from the experiment editor (on the left side of the main window). The region viewer panel, which was empty before, will be updated with the region's image. The **Region Viewer** is shown in the next screenshot:

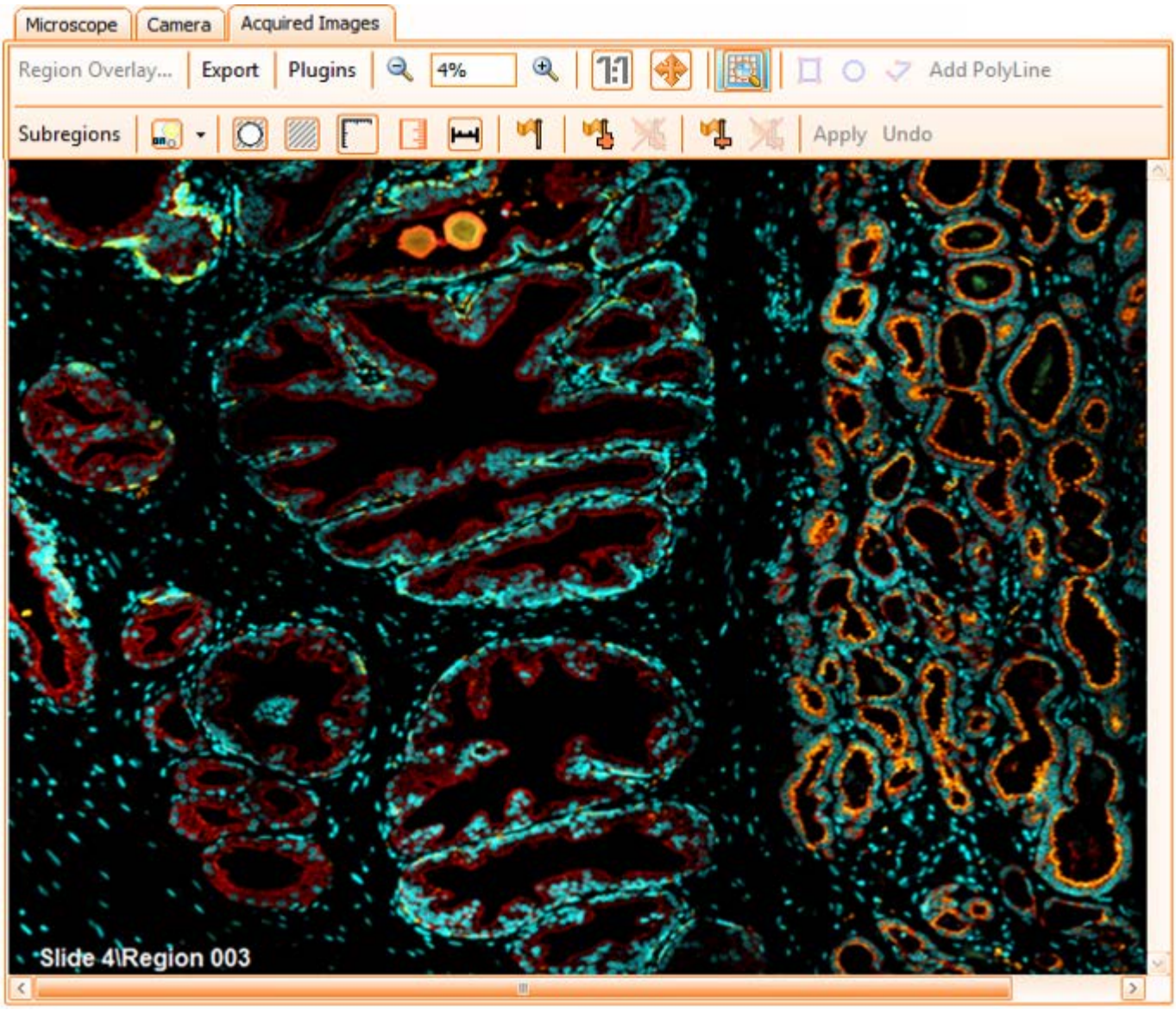


Figure 199 - Acquired Images viewer (Fluorescence experiment)

The slide name and region name are displayed at the bottom left corner of the region viewer.

In **fluorescence** experiments, the button is enabled. Clicking on it yields a new window, as shown below:

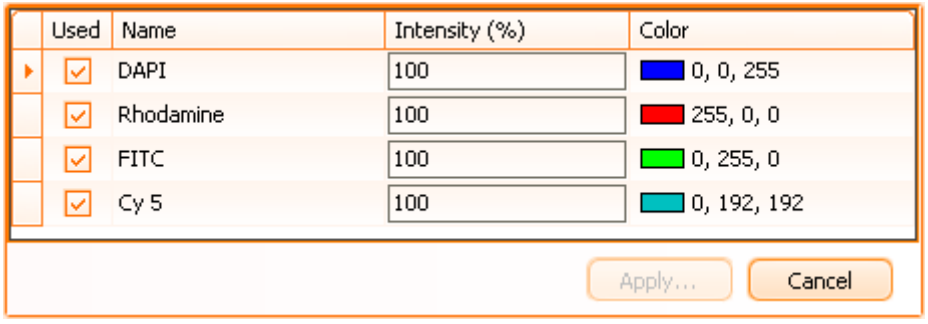


Figure 200 - Adjusting channel intensity and color

This window allows you to choose which channels to view in your acquired image. Here, you can adjust the color and light intensity for each channel. If more than one channel is selected, clicking **Apply** will yield an overlay image, which is composed of the selected channels according to the set algorithms.

In **brightfield** experiments, the **Region Overlay…** button is disabled because there is only one image for each Field Of View.

Zoom in and **Zoom out** buttons allow zooming the image. Another option would be to type the desired zoom value in the zoom editor. Keep in mind, however, that the Region Viewer is designed to give the user an overview of the entire region and therefore is limited in giving detailed magnified images.

After a region is acquired, the user can double press the left mouse button on the region to open the **Acquired Image** tab and to see the acquired image splitted into Fields of View.

The **toolbar** contains the following buttons:

- Export: **Fields of View**, **Region Overview**;
- Plugins;
- Zoom in;
- Zoom out;
- Map: shows/hides region map;
- Add subregions and tissue areas belonging to categories;
- Subregions: **Show Defined Regions**, **All Categories**, **Manage Categories**;
- Crop (it marks the images that have been acquired outside the defined region);
- Gridlines: default value is **Off** ;
- Scalebar: default value is **Off**;
- Measure;
- Annotations;
- Illumination Correction;
- Reacquire Flag;
- Add Flag (it adds Fields of View to the region);
- Remove Flag (it removes Fields of View from the region);
- Time lapse acquisition button (if it is the case).

To view a more detailed, high resolution portion of the acquired image, double click on it. This will create a new window called the **Field of View Viewer** (see next section).

5.17.1 Subregions

Subregions are normal regions defined within the Region Viewer, having the same properties as the regions from the Slide Preview. You can see them in the experiment editor and they can be acquired just like normal regions. The advantage of the subregions is to ensure higher flexibility and more detailed analysis on a more detailed area of tissue.

To add subregions, go to **Region Viewer Toolbar** → **Subregions** →**Show Defined Regions** (only **Show Defined Regions** should appear checked).

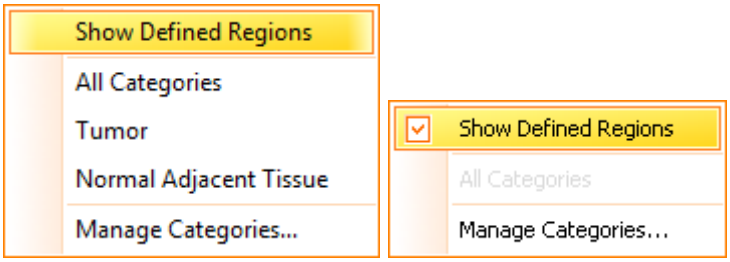
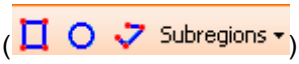


Figure 201 - Add Subregions context menu

Now, choose the subregion shape from the toolbar (

). Then add the desired subregion on the viewer using the mouse, just like for any normal region (see **Chapter 5.16.1**).

To be able to see all subregions you have added, simply check **Show Defined Regions** option from the **Region Viewer Toolbar.**

5.17.2 Categories

Sometimes, you may want to emphasize certain small areas on your region that could contain high interest information for your research. These areas can be exported for analysis or they can be used as a highlight tool.

For instance, a tissue may contain both tumor areas and normal adjacent tissue (non-tumor) areas. For each type of area (e.g. tumor, non-tumor) you may create a **category** in order to highlight that particular area on the image.

Using categories might help you in order to perform a precise analysis.

Add categories

In order to add categories, go to **Region Viewer Toolbar** → **Subregions** → **Manage Categories…** A dialog will appear (containing already added categories, if existing) where you can add new categories, giving each one a name (mandatory) and assigning a color. The list of categories is per project, not per region.

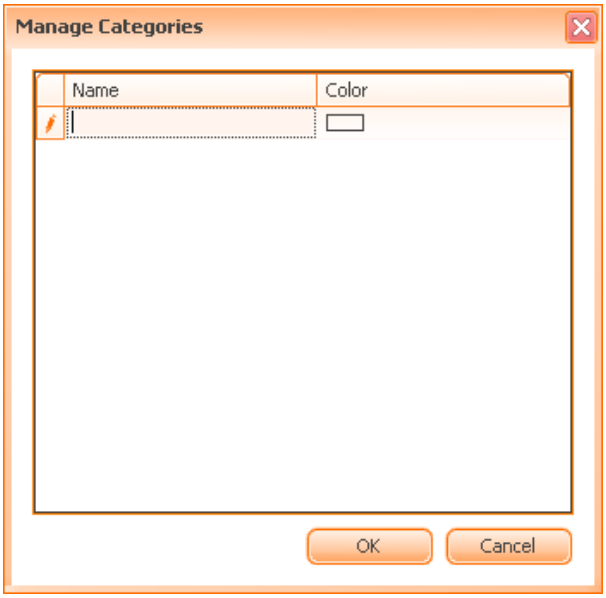


Figure 202 - Add categories dialog

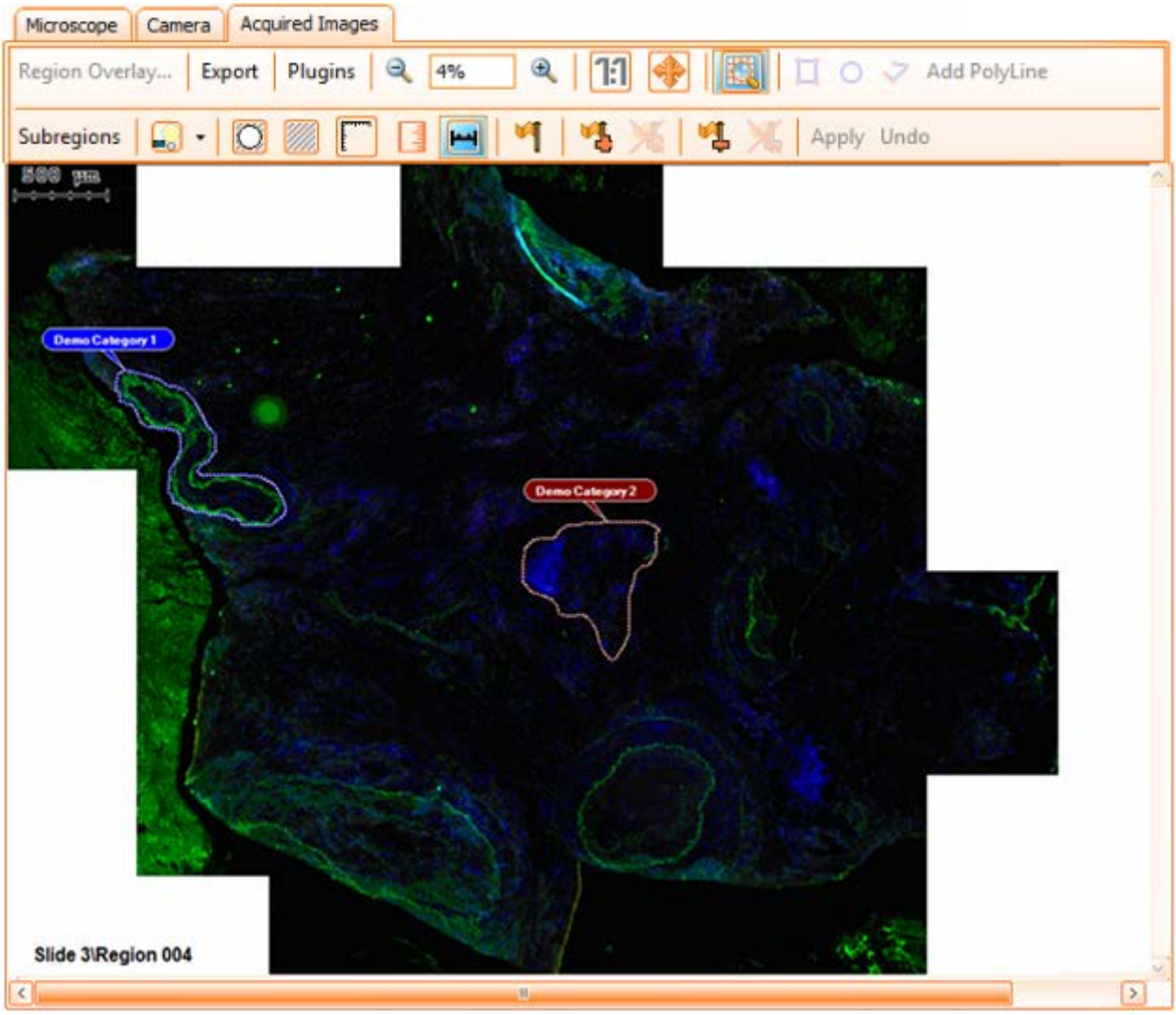


Figure 203 - Region Viewer: example of categories

You may also use the categories when you are about to export images. For further details please see **Chapter 5.20** of the current manual.

Note - All existing categories are listed in the **Subregions** menu.

Remove categories

In order to perform this action, go to **Region Viewer Toolbar** → **Subregions** → **Manage Categories…** and select the category you want to remove, then press the **Delete** key from your keyboard.

Apply categories on regions

To create these areas, go to **Region Viewer Toolbar** → **Subregions**, then select only the desired category.

Now, choose the area shape from the toolbar.

Figure 204 - Toolbar: categories shapes

Then add the desired area on the viewer using the mouse, just like for any normal region (see **Chapter 5.16.1**).

Once created, these areas cannot be modified, only removed. To remove the areas, right click inside the desired area and then choose **Remove [*area name*]…**

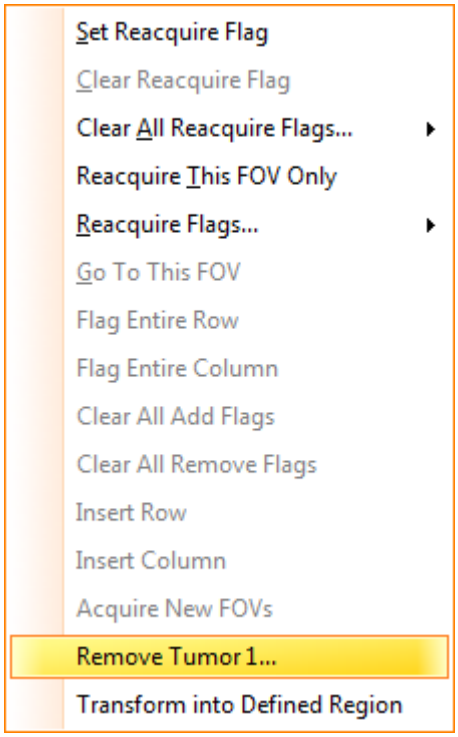


Figure 205 - Remove category area context menu

View / hide categories on regions

To be able to view all tissue areas belonging to a certain category, check only that particular category in **Region Viewer Toolbar → Subregions**.

If you want to see all existing categories, check **All Categories** option from **Region Viewer Toolbar → Subregions**.

To hide the tissue areas belonging to a category, simply uncheck that particular category in **Region Viewer Toolbar → Subregions**.

Export tissue areas belonging to categories

- When exporting the **Region Overview**, you have the possibility to choose an option that also exports the categories.
- When exporting the **FOVs images**, you have the possibility to choose an option that only exports the images belonging to the respective category.

For more details please see **Chapter 5.20** of the current manual.

Transforming regions from categories into subregions for reacquisition

If the user needs to reacquire the tissue area from a category, it is possible to transform it into a **Defined Region** and then perform reacquisition.

In the region viewer, right click on the border of a category and from the contextual menu choose **Transform into Defined Region** option. The category will transform into a **Defined Region**, it will disappear from the region viewer and it will be visible in the experiment editor, so it can be reacquired.

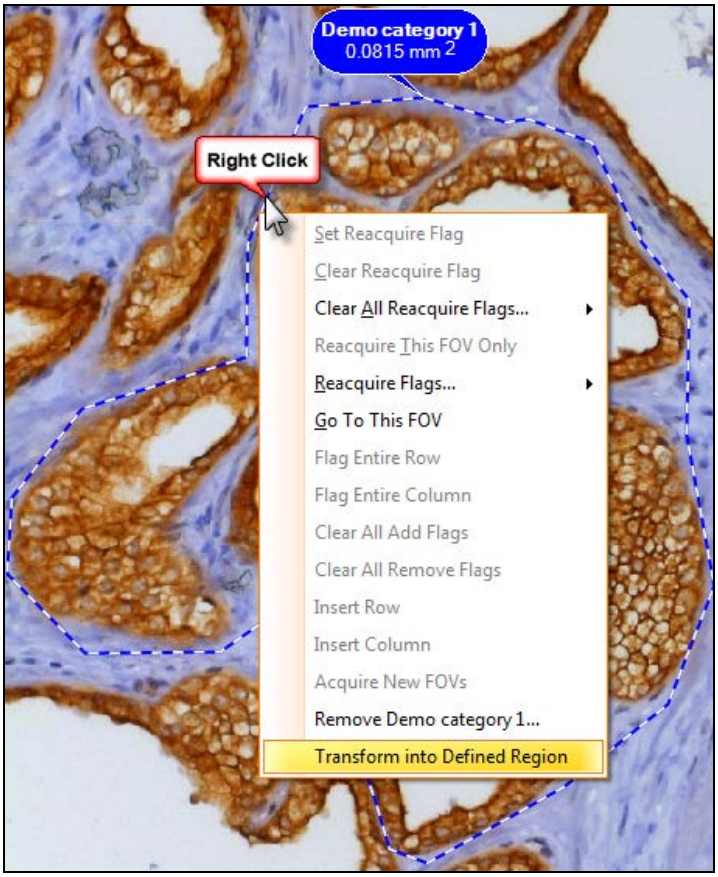


Figure 206 - Transform region from a category into a subregion for reacquisition

5.17.3 Annotations

Press the **Subregions** (Subregions) button in order to access the **Subregions** contextual menu.

The following options will be available:

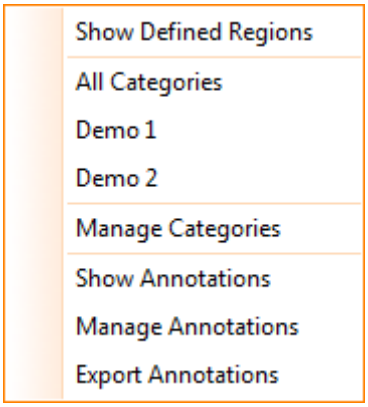


Figure 207 - Annotations contextual menu

Show Annotations

To add annotations, go to **Region Viewer Toolbar** → **Subregions** →**Show Annotations** (only **Show Annotations** should appear checked).

Now, choose the annotation shape from the toolbar. Then add the desired shape on the viewer using the mouse, just like for any normal region (see **Chapter 5.16.1**).

To be able to see all annotations you have added, simply check **Show Annotation** option from the Region Viewer toolbar.

PolyLine Measurements

This feature allows drawing a line shape in order to measure a certain area.

Press **PolyLine** button and draw desired shape on the sample (see **Chapter 5.20** for more details).

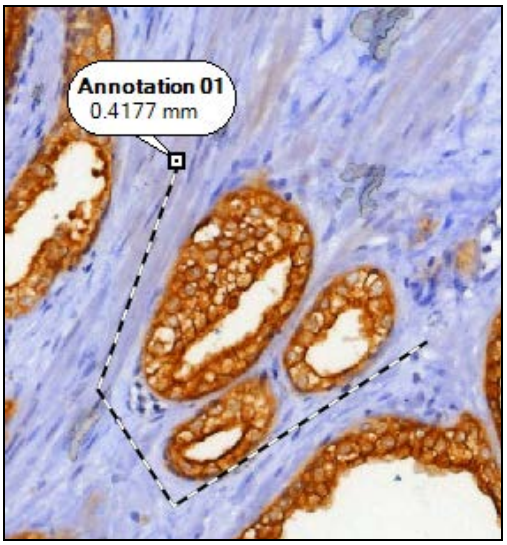


Figure 208 - Drawing PolyLine

Manage Annotations

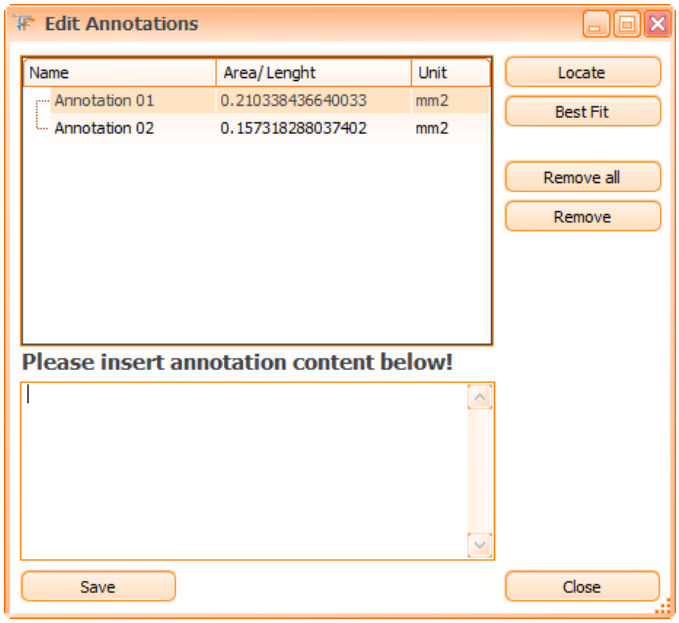


Figure 209 - Edit Annotations dialog

- **Locate**: by pressing this button, the selected annotation will be located on the sample and displayed at the current size of the sample.
- **Best Fit:** by pressing this button, the selected annotation will be located on the sample and displayed at the current size of the region viewer.
- **Remove**: removes the selected annotation
- **Remove all**: removes all existing annotations.
- **Please insert annotation content below**: write desired content for the respective annotation.

Edit annotation directly on the sample

An annotation can also be edited directly on the image viewer: it can be renamed, its content can be changed or it can be removed. To edit an annotation you must use the little black framed square on the contour of the annotation. If pressed, this little square will display an edit box, where you can operate the following actions:

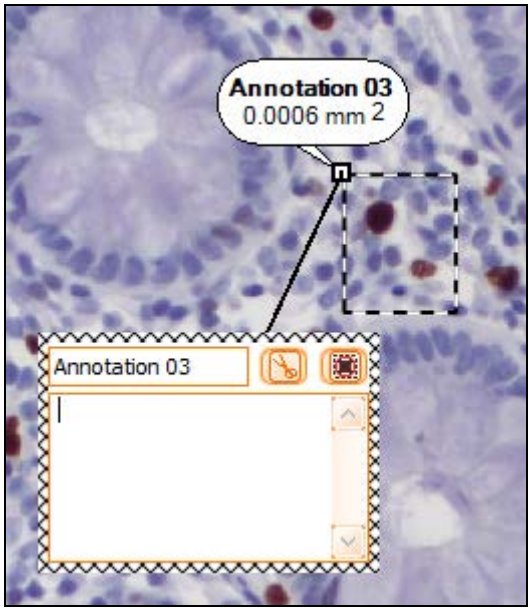


Figure 210 - Edit annotation directly on the sample

- Edit the name of the annotation: write the desired name in the upper-left corner of the box, then press **Enter** key to save the changes;
- Write a comment in the space below the name, you can also erase the existing comments (all the changes concerning the notes are automatically saved);
- Delete the annotation by pressing button;
- Close the edit box by pressing button.

Export Annotations (please see **Chapter 5.20.5**)

5.17.4 Illumination Correction (Shading Correction)

Occasionally, on the acquired images, some shades may appear. They can be caused by imperfections of any component of the lightpath, specks/impurities on the camera/objective.

TissueFAXS allows you fixing such shading problems by using the **Illumination Correction** function. In this chapter, you will find how to use this feature.

Note - This operation is available only for **brightfield** experiments.

You can access the **Illumination Correction** menu by pressing the **Illumination Correction** button ( ) from the **Region Viewer** control.

To apply illumination correction, a *correction image* is required (mandatory).

The **correction image** is an image computed in order to store information about the shades in the light path. By applying this image to a certain region, the shades will be eliminated and the images will be uniformly illuminated.

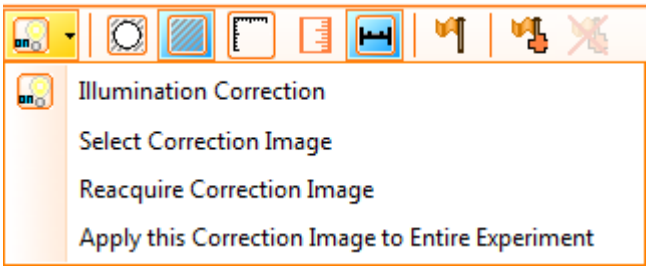


Figure 211 - Illumination Correction menu

The **Illumination Correction menu** contains the following items:

- **Illumination correction**: choose this option to automatically apply the correction image to your region. If there is no correction image available, you will receive a message box that offers you three possibilities:
  - **Compute** a correction image, using the already acquired images; this is the solution when a correction image is not available and reacquiring the correction image with the same settings/hardware as the region is not possible anymore.
  - **Acquire** a correction image (this is equivalent to **Reacquire correction image** from the **Illumination correction** menu);
  - **Cancel** the whole process of illumination correction.

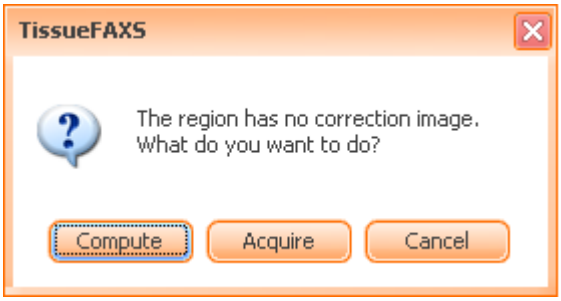
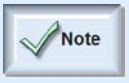


Figure 212 - Compute correction image message box

- **Select correction image**: by choosing this option, you will be displayed a dialog where you can choose from the listed regions (containing correction image) the one you want as correction image.



- The region that appears in *red* in this dialog is the currently opened region

After selecting the respective image, you can effectively apply the illumination correction.

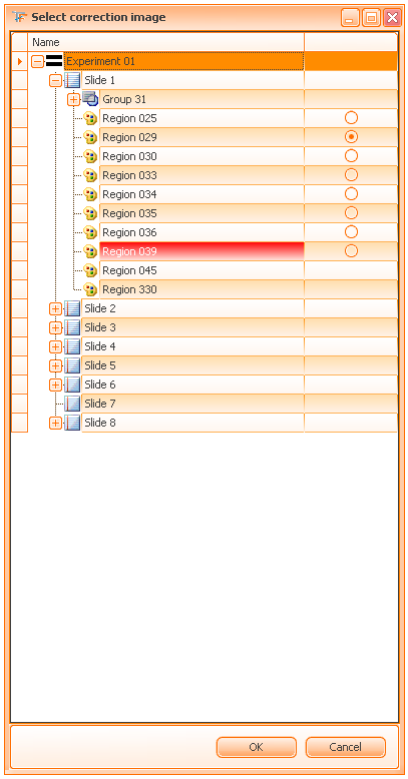


Figure 213 - Illumination Correction: Select correction image

- **Reacquire correction image**: this option should be used when you wish correcting the illumination for an already acquired region. The following panel will appear:

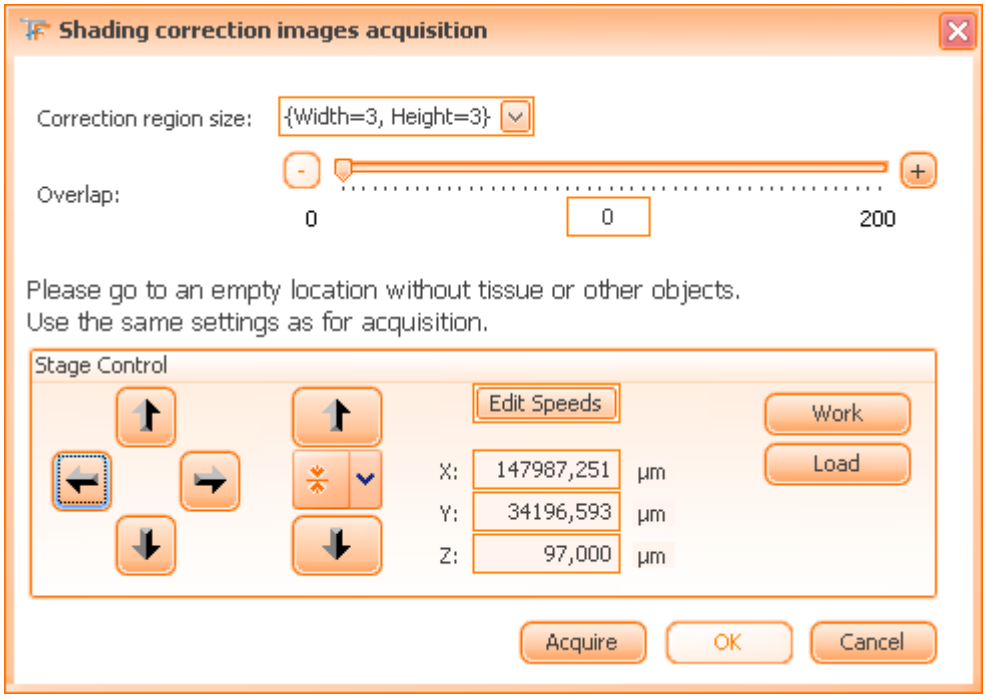


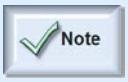
Figure 214 - Acquire correction image control panel

- **Correction region size**: you can specify here the width and the height, expressed in FOVs, of the area used to compute the correction image. Generally, a large area size is to be preferred, but this is not mandatory.
- **Overlap**: the overlap parameter helps you control the size of the overlapping area shared by neighboring FOVs that form the correction region. You can adjust the

number of pixels that can be shared (overlapped) by two FOVs by using the slider. Usually, by increasing the value, you can obtain a better correction image, and thus, a better illuminated region.

- Press **OK** button to save the correction image or **Cancel** button to cancel the acquisition process.

- **Apply this correction image to the entire experiment**: the correction image of the current region from the region viewer will be applied to all regions of an opened experiment.



- Each time, before starting the acquisition process, you will be displayed an **Acquisition Information** dialog where you can check the **Acquire correction image** option in order to obtain a correction image for the respective region. **TissueFAXS** will use the same acquisition settings for the region and for the correction image.

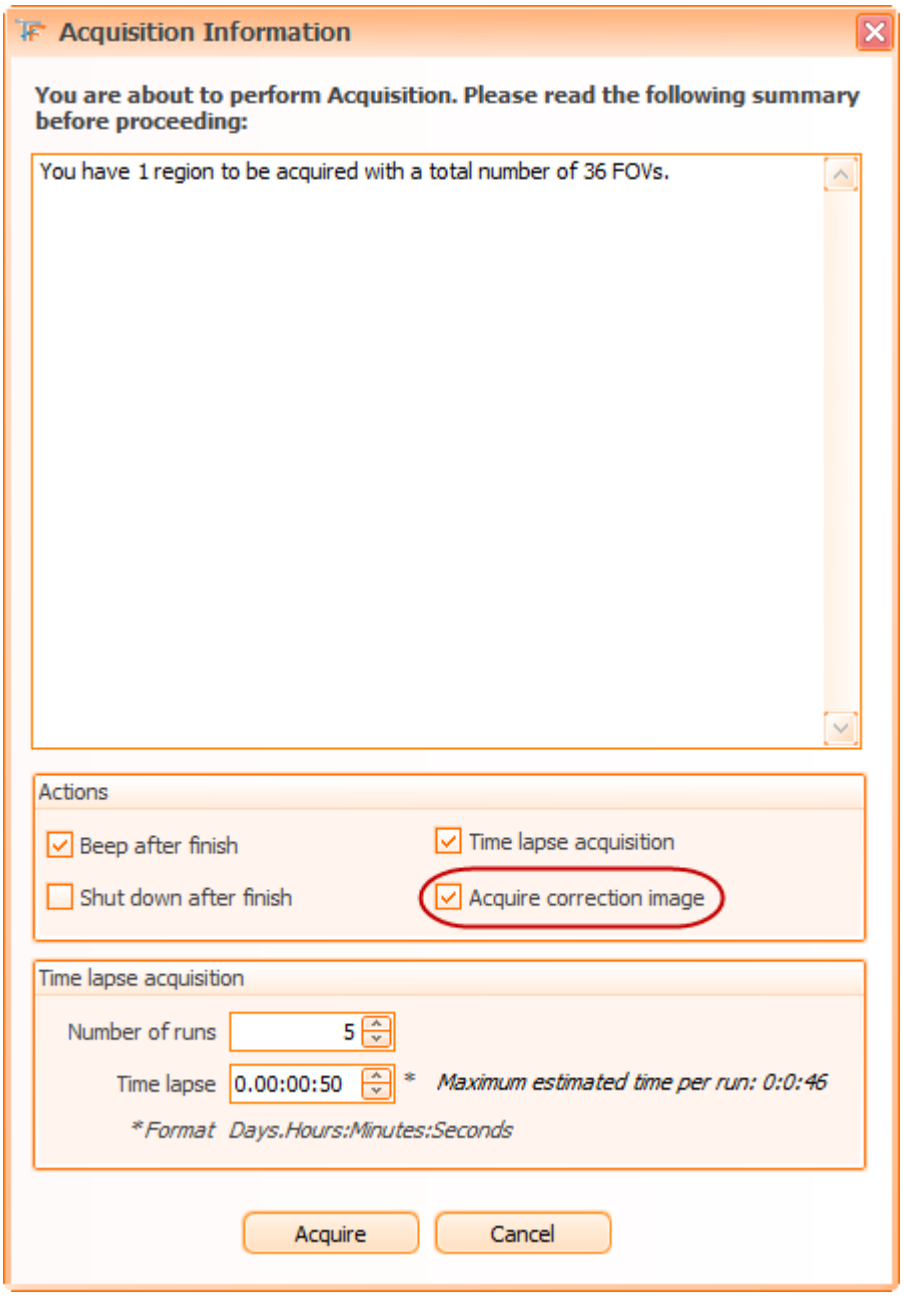


Figure 215 - Acquisition Information : Acquire correction image checkbox

The Shading correction images acquisition dialog will appear (see above the *Reacquire correction image* paragraph).

TIP - If you frequently acquire correction image you can set this option as default from **Tools → Options → Application Options →Remember**.

5.17.5 Flags

In **TissueFAXS**, the **flag** option is used in the reacquisition of the FOVs, when the initial acquisition process has generated unclear or unsatisfying results.

By using the flags, you can modify the shape of your region by adding or removing FOVs.

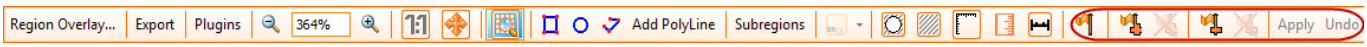


Figure 216 - Region Viewer Toolbar

Reacquire Flags

In order to view and manage the flags, you must first ensure the **Set Flag** button ( ) from region viewer toolbar is pressed ( ).

To add flags you have two options:

- Directly left-clicking on the FOV you choose for focus;
- By pressing **Set** "**Reacquire Flag**" from the contextual menu that appears when right clicking on the FOV chosen for focus.

For both adding flags methods, the selected FOV will be set as a focus point and all its neighboring FOVs will be selected for reacquisition as well. The size of the neighboring area is the size specified by **Tools → Options → Scan Settings → Focus → Focus Strategy Neighbors**.

To remove flags you have three options:

- Directly left-clicking on the FOV;
- By pressing **Clear** "**Reacquire Flag**" from the contextual menu that appears when right clicking.
- By pressing **Clear All** "**Reacquire Flags**"**…** from the contextual menu that appears when right clicking and then choosing one of the three available options: **For Current Region**, **For Whole Slide**, **For Entire Experiment**.

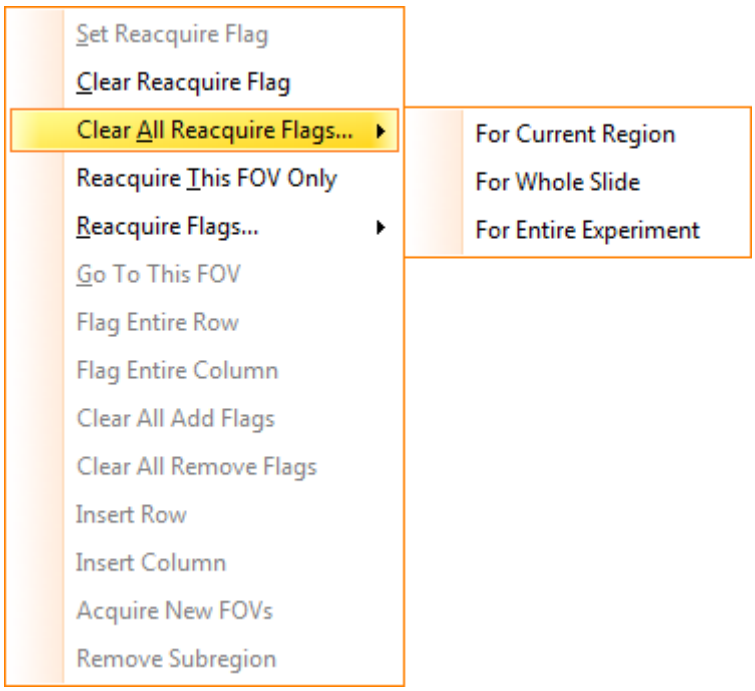


Figure 217 - Clear All "Reacquire Flags" context menu

Note

- For the first two **remove flags** options, you may notice that by clicking on the focus flag, all neighboring flags will be removed (including the focus flag itself). However, by clicking on a neighboring flag (non focus flag), only this flag will disappear.

Reacquire FOVs

- If you choose the **Reacquire This FOV Only** option, only the current selected FOV will be acquired.
- If you choose the **Reacquire Flags** option, you have three choices: **For Current Region**, **For Whole Slide**, **For Entire Experiment**.

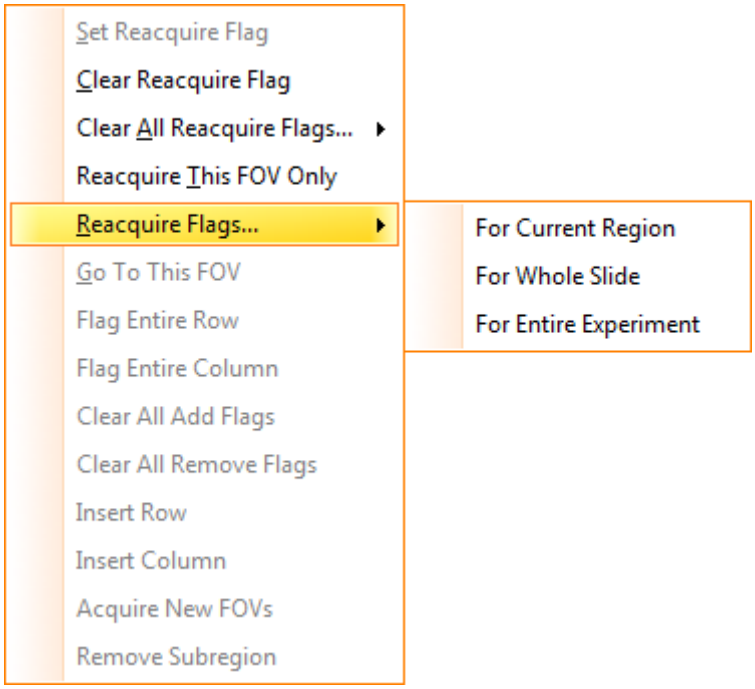


Figure 218 - Reacquire Flags contextual menu

Add Flags

By adding flags, the size of the region will be enlarged in order to include new tissue area that could be relevant for analysis.

As previously mentioned, **FOV Matrix Size** property of the region represents the number of FOVs per row and column. Based on the shape of the region, a part of these FOVs are not acquired.

Adding flags without changing the FOV matrix size property:

- Check the **Add flag** button ( ), then click on an empty position outside the region shape you might be interested in (next to already existing FOVs) but inside the crop area. To remove the added flag, click again on the same empty position. After finishing this operation, uncheck the **Add Flag** button.

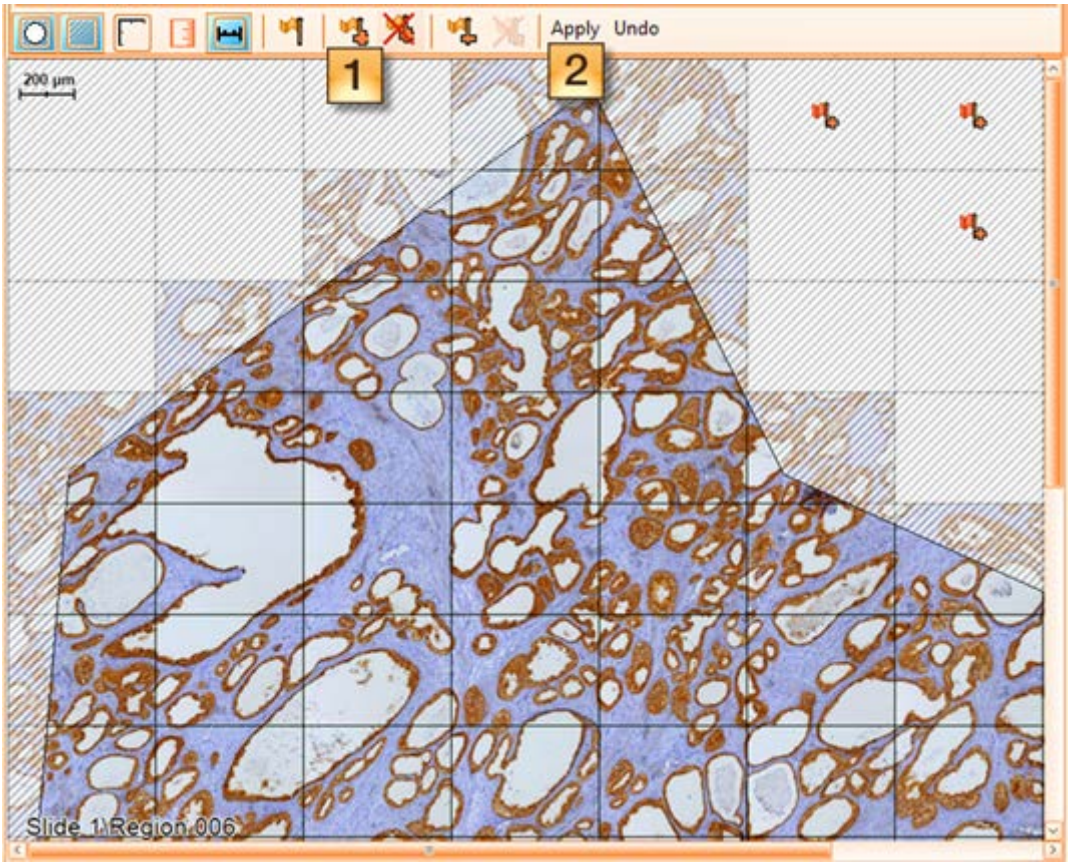


Figure 219 - Adding flags without changing the FOV matrix size (a)

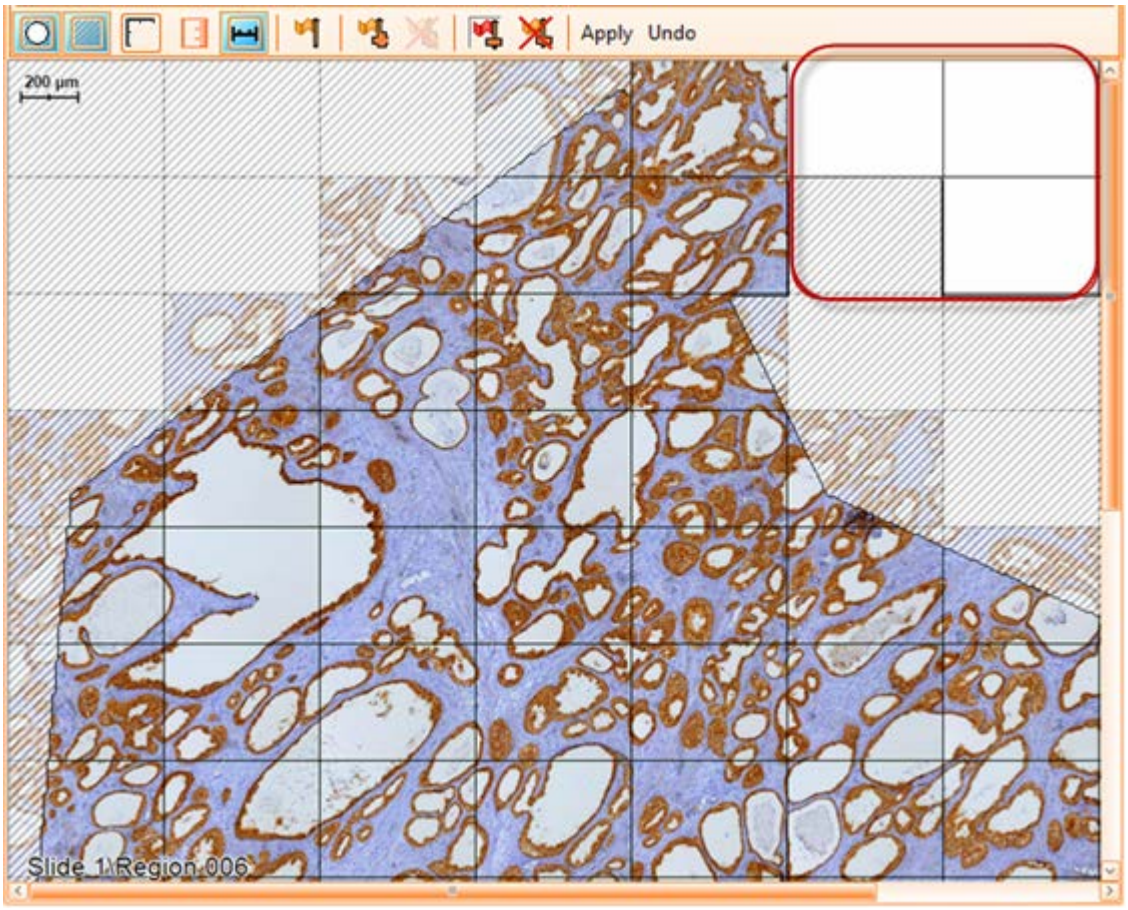


Figure 220 - Adding flags without changing the FOV matrix size (b)

Adding flags by changing the FOV matrix size property:

- If you click on the first/last row, you can select Insert row before/after;
- If you click on the first/last column, you can select Insert column before/after;
- Check the **Add flag** button ( ), then click on an empty position outside the region shape you might be interested in (next to already existing FOVs). To remove the added flag, click again on the same empty position. After finishing this operation, uncheck the **Add Flag** button;
- You can also click **Flag to add entire row/column** from the contextual menu that appears by right clicking on the newly inserted row/column. To reverse this operation, choose **Unflag entire row** from the contextual menu;

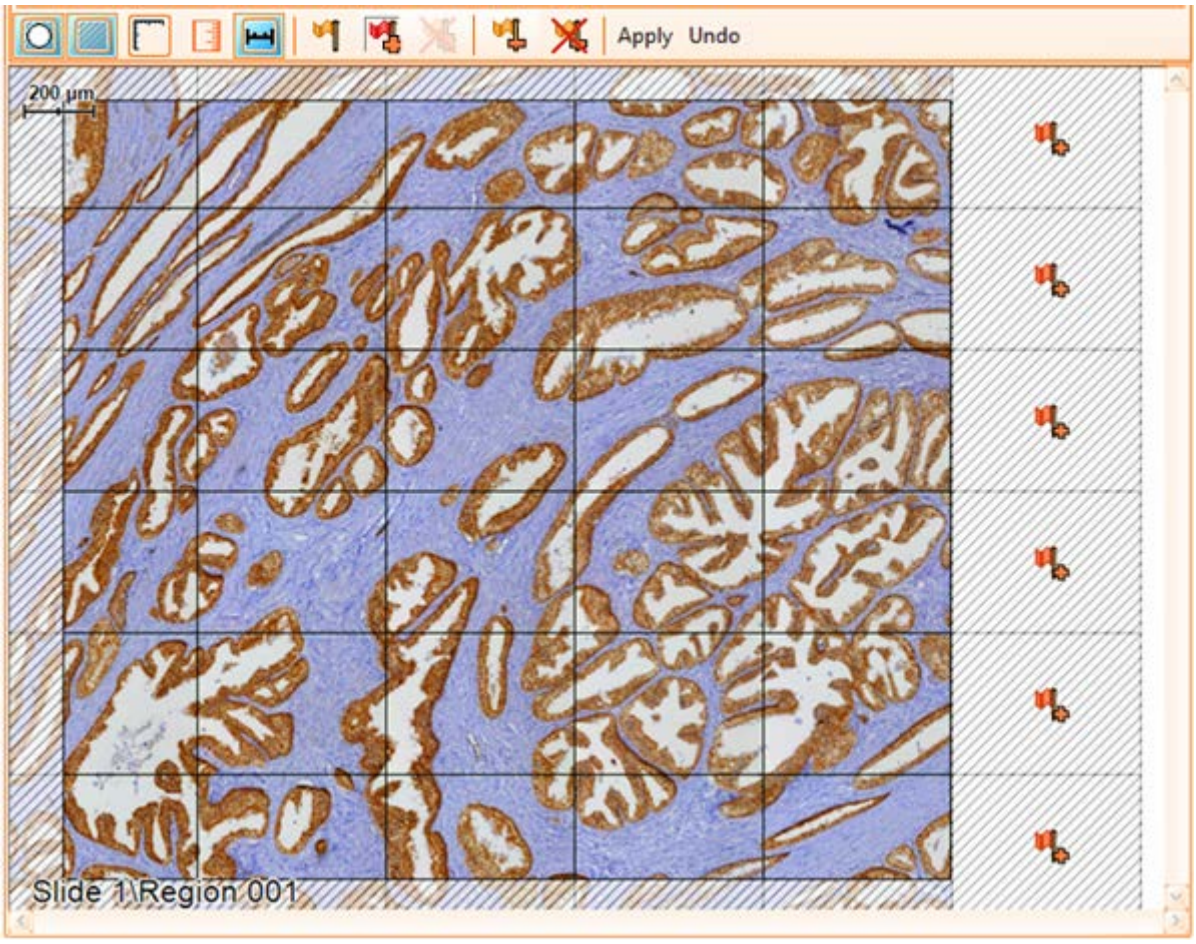


Figure 221 - Adding flags by changing the FOV matrix size (a)

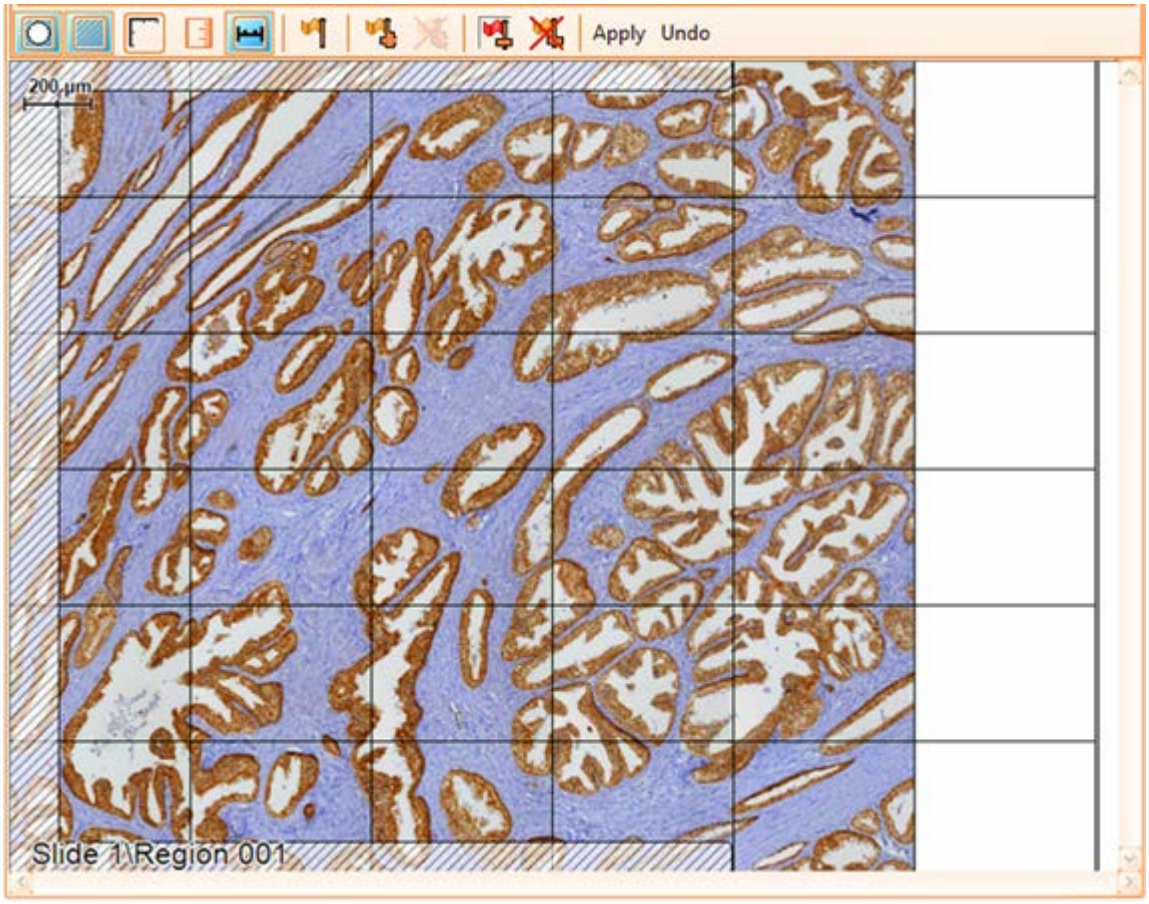


Figure 222 - Adding flags by changing the FOV matrix size (b)

- To remove all added flags, click **Clear** "**Add Flags**" ( ) from the Region Viewer toolbar or choose **Clear** "**All Add Flags**" from the contextual menu.

Remove Flags

- Check the **Remove flag** button ( ), then click on a border FOV you don't need. To uncheck the added flag, click again on the same FOV. After finishing this operation, uncheck the **Remove Flag** button.

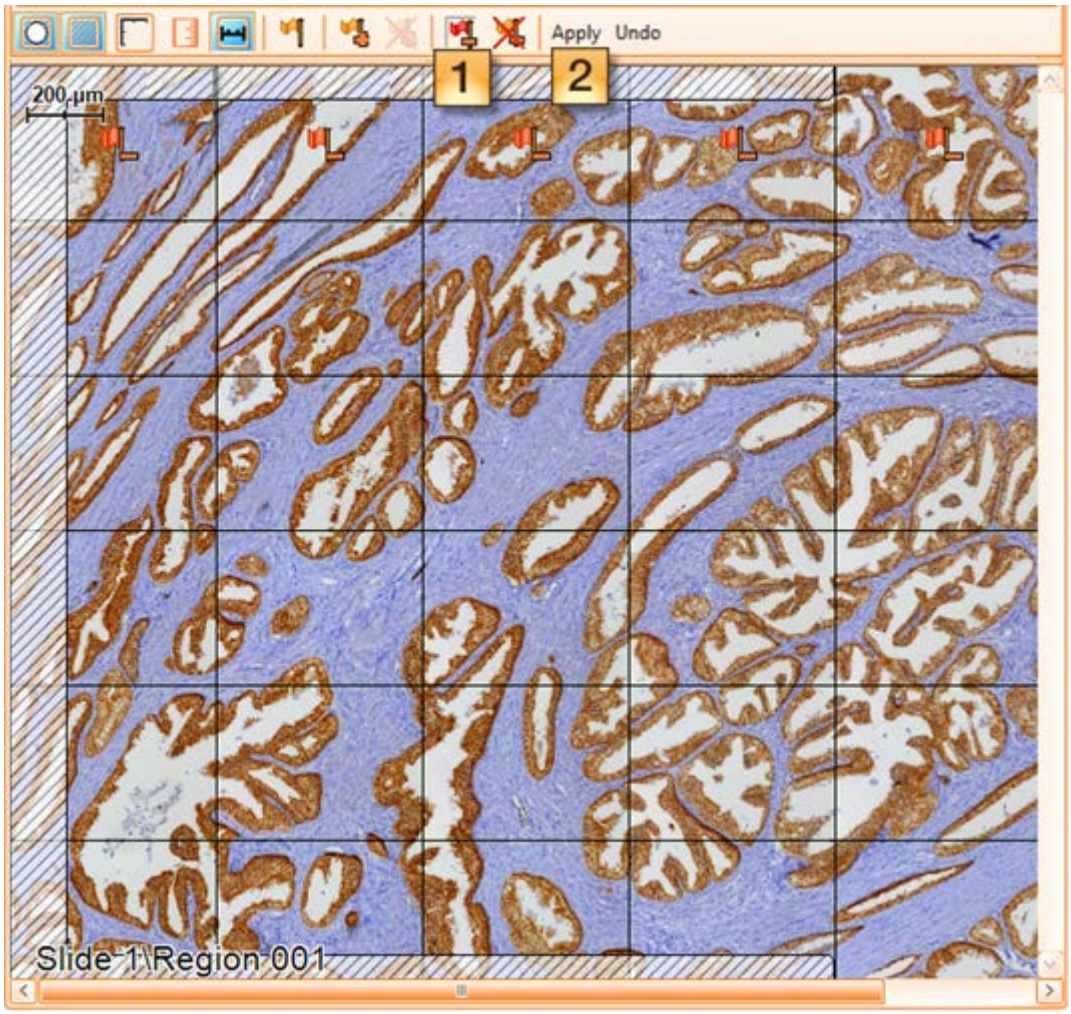


Figure 223 - Remove flags (a)

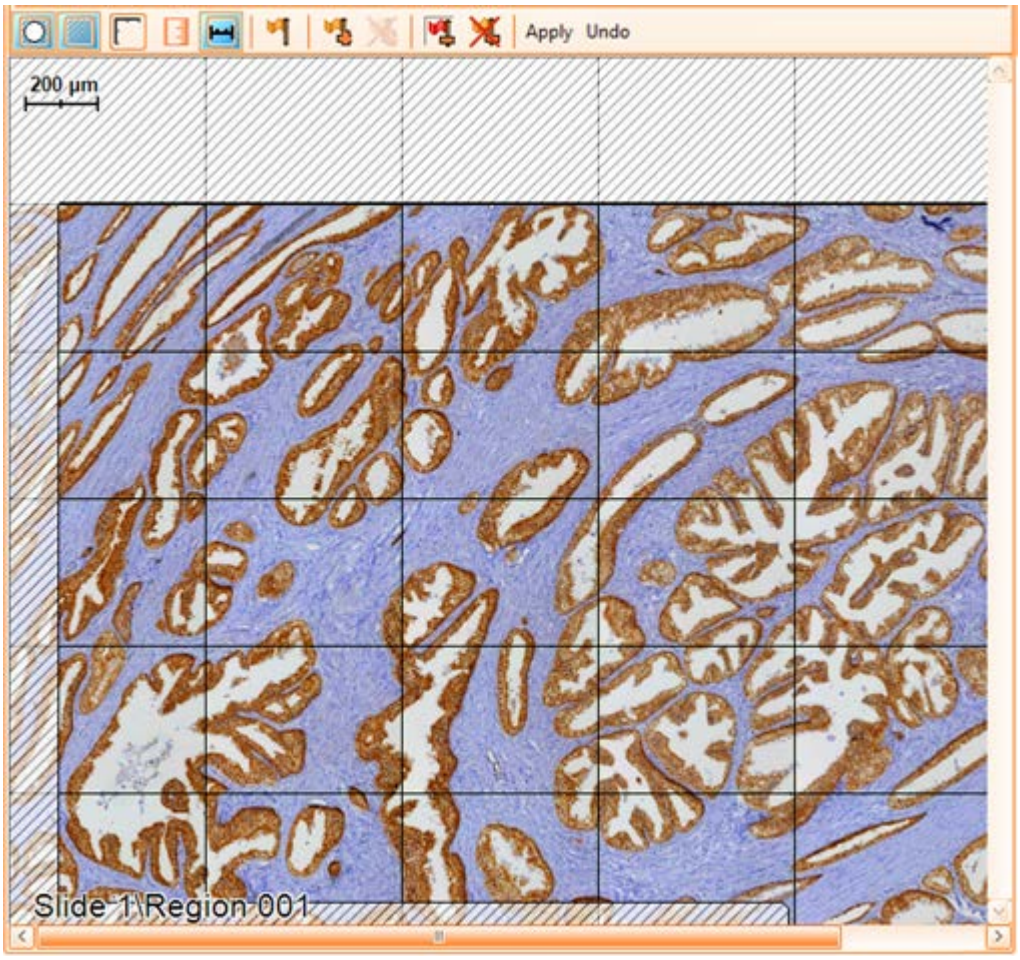


Figure 224 - Remove flags (b)

- You can also click **Flag to remove entire row/column** from the contextual menu that appears by right clicking on the border row/column. To reverse this operation, choose **Unflag entire row** from the contextual menu.

- To remove all added flags, click **Clear** "**Remove Flags**" ( ) from the Region Viewer toolbar or choose **Clear** "**All Remove Flags**" from the contextual menu.

Apply

Pressing the **Apply** button (

) will effectively modify the region shape by adding/removing FOVs (will remove images files too).

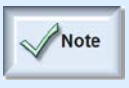
Undo

Pressing the **Undo** button (

) will discard all changes (insert rows/columns, **Add/Remove Flags**).

Acquire New FOVs

This option from the contextual menu will acquire all newly added FOVs.



- The **Flags** feature is not compatible with the **Time Lapse** acquisition and therefore it is available only for **Standard** acquisition mode.

5.18. Field of View Viewer

To view a detailed, high resolution section of an acquired image, double click on it within the **Region Viewer** window. This will create a new window called the **Field of View Viewer (FOV Viewer)**.

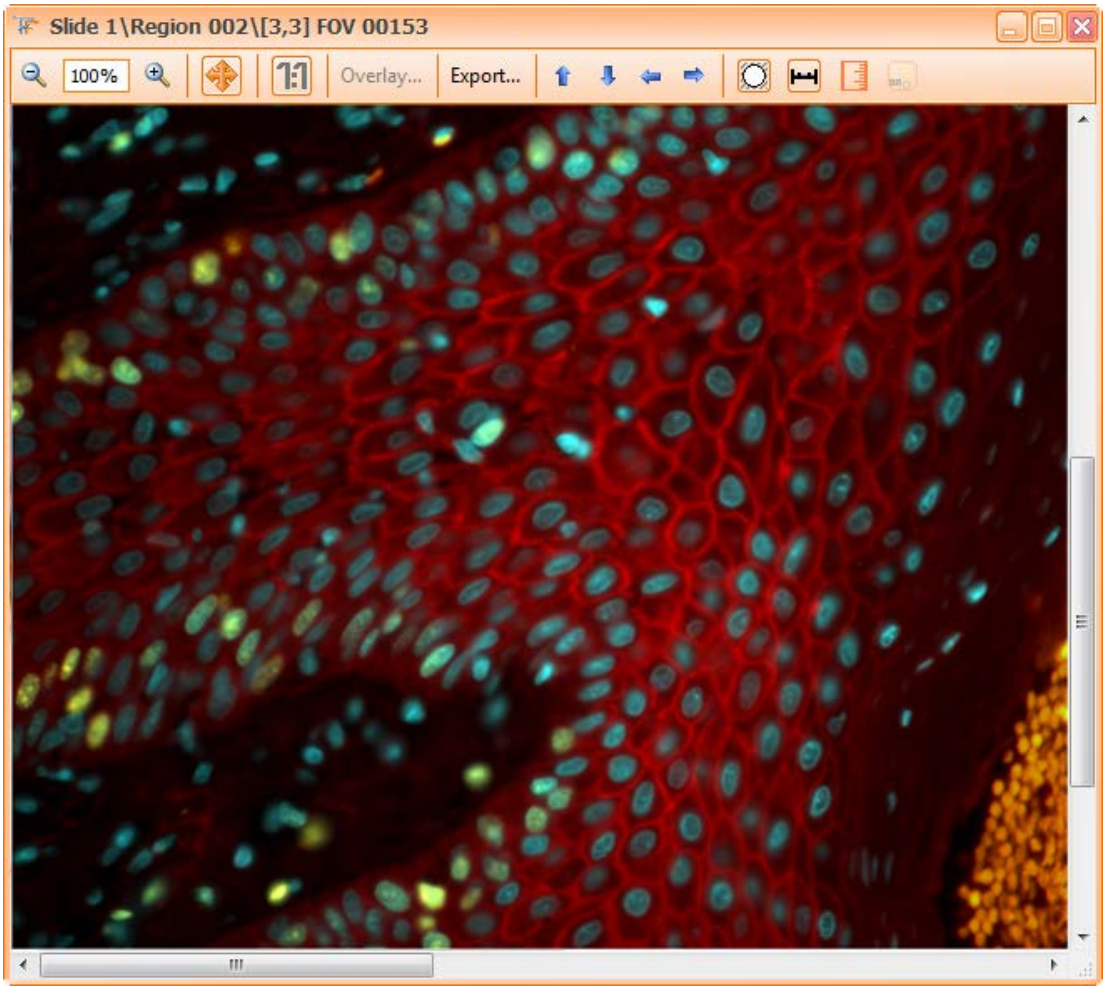


Figure 225 - FOV Viewer (Fluorescence Experiment)

The features in the **FOV Viewer** are similar to those of the **Region Viewer**. The difference is that the **FOV Viewer** gives higher resolution images because it displays the original acquired image directly (and not just a thumbnail image, as with the **Region Viewer**).

The user can navigate to neighboring FOVs with the arrow buttons at the top right corner of the viewer: .

TIP

- The FOV displayed will be highlighted within the **Region Viewer**.
- In the eventuality that the image does not fit the screen, drag it by using the hand tool instead of scrolling up/down or left/right. To do this, hold the right mouse button down and drag the image, then release the mouse button to stop.
- Similar with the region viewer, use the trackbar to see the FOV at a certain moment by moving the cursor (

). This option is available only if the FOV was acquired with time lapse (for details please see Chapter 0).

5.19. Field of View Viewer - Overlay feature (Fluorescence)

The **Overlay…** button in the **FOV viewer** button displays the same dialog for adjusting channels intensities and colors as for the **Region Viewer** (see previous section).

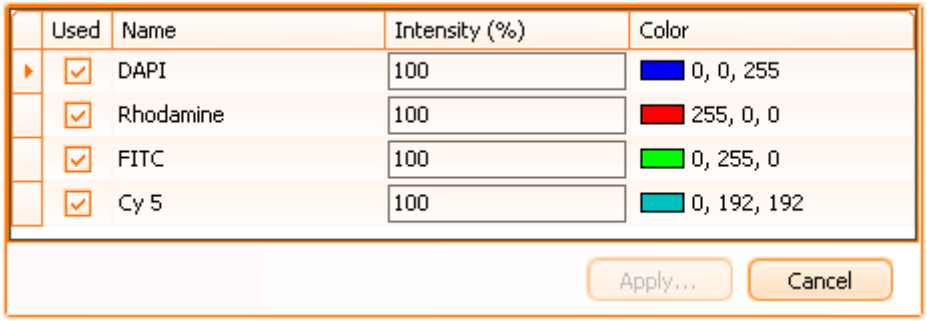


Figure 226 - Overlay Settings for a single FOV

The difference is that the **Apply** button can work on multiple items as shown in the image below:

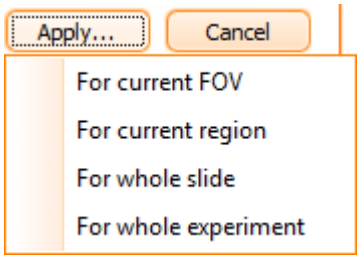


Figure 227 - Apply overlay menu

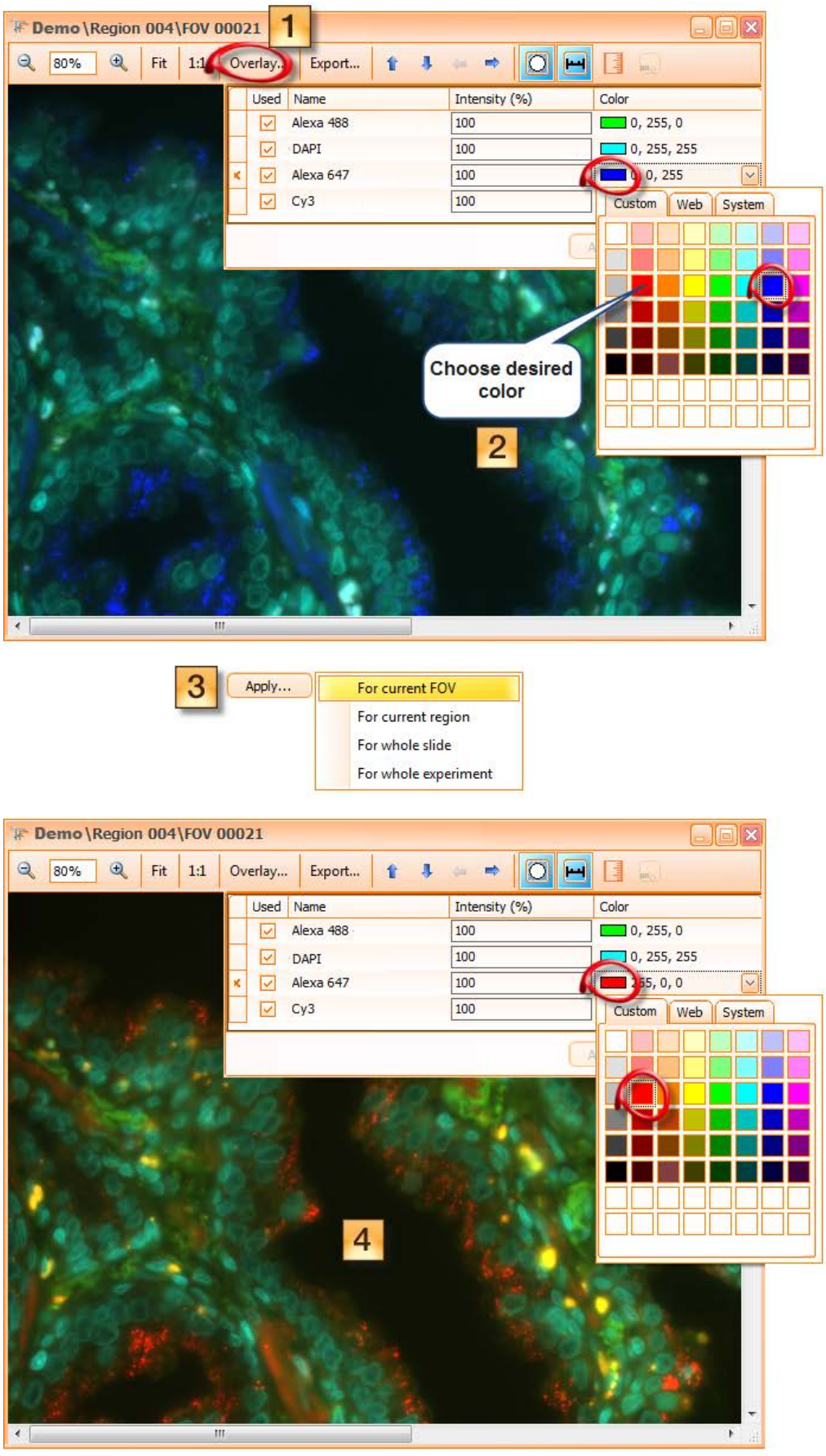
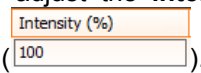


Figure 228 - FOV Overlay - Example of adjusting color for one channel

The user can also adjust the **intensity** for each channel, by typing the desired value in the corresponding field (

).

5.20. Export

TissueFAXS allows exporting acquired images as a complete region overlay or image by image.

In certain cases *csv* files can also be exported.

5.20.1 Export FOVs Images

You can access this option in two ways:

- **Region viewer → Export → Fields of View**;

Figure 229 - Export FOVs Images Option (a)

- **Experiment Editor → Region Context Menu**.

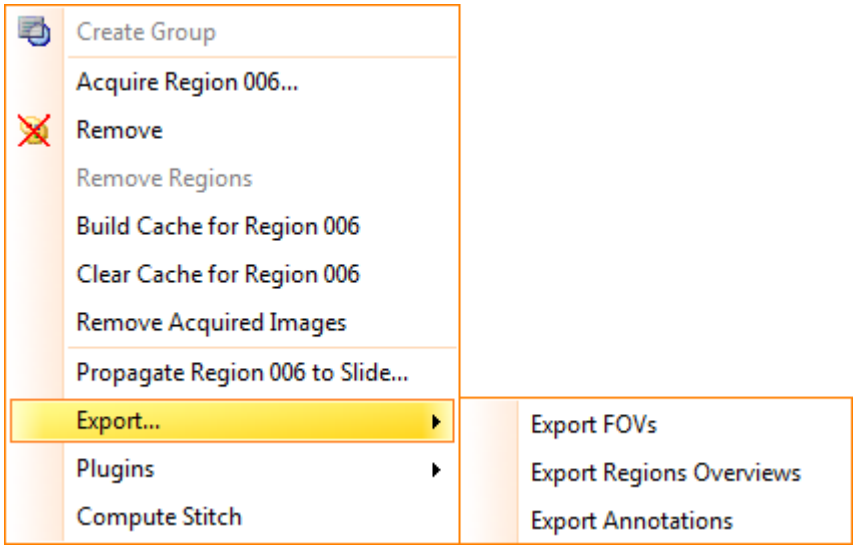


Figure 230 - Export FOVs Images Option (b)

- If you want to export more regions or FOVs simultaneously, you can select multiple items from the Experiment Editor tree.
- You can also select Experiment item from Experiment Editor tree to export all the regions or FOVs.

In both cases, the following dialog will appear:

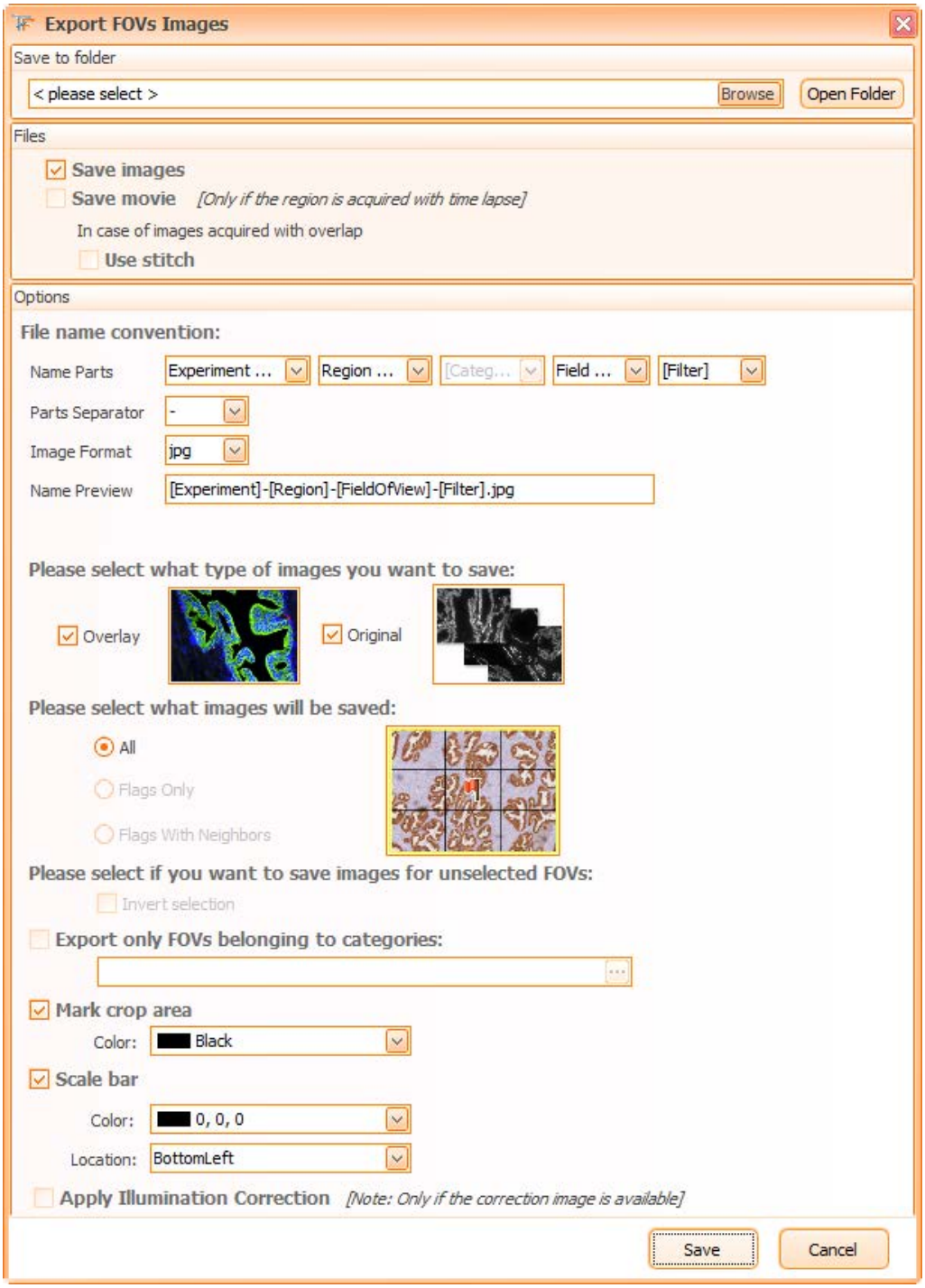


Figure 231 - Export FOVs Images dialog

TissueFAXS allows you configure the export as you desire, by choosing exactly the contents that is of interest to you. In the dialog displayed above, you can adjust the following **settings**:

- Select the **storage folder**;
- Select **files** to be exported:
  - Images
  - Movies (option available only if the region was acquired with time lapse)
  - Use stitch
- Select the **file name convention** (Name Parts, Custom Part, Parts, Image Format, Name Preview);

- Select the **type of images** you want to save:
  - **Overlay -** The FOV images will be composed from all the channels as currently specified in region viewer.
  - **Original** - The FOV images will be exported separately for each channel as they were acquired.
- Select **what images will be saved**:
  - **All**;
  - **Flags Only** and **Flags with Neighbors** (these options are available only if you have at least one flag set).
- **Invert selection** - Only the FOVs not marked with flag or neighbors of the flagged FOVs (applies if **Flags with Neighbors** is selected) are exported;
- **Export only FOVs belonging to categories**: in this case, only the FOVs that belong to the selected categories are exported, by pressing the browse button ( ).

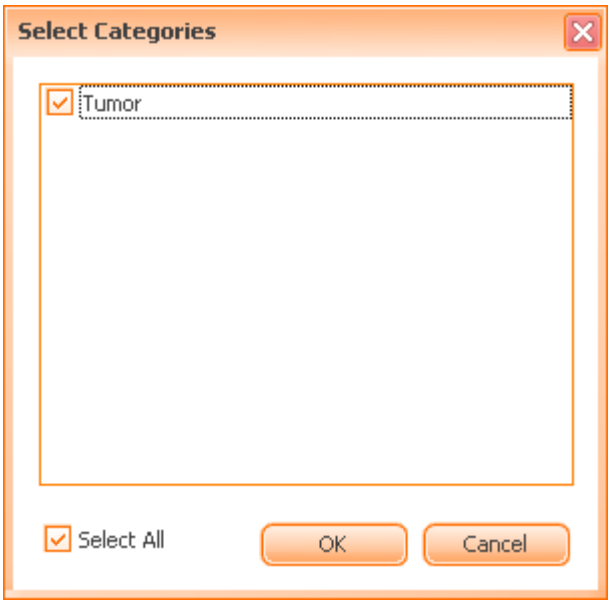
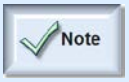


Figure 232 - Export FOVs: Categories included



- In the selection list, only those categories will be listed that contain tissue areas on their overview image.

- **Mark Crop Area**: it marks the contour of the region for FOVs near the region border; you can also select its color;
- **Scale bar**: please see **Chapter 4.1.2** of the current manual.
- **Apply Illumination Correction**: this option is enabled only for **Brightfield** experiments. The correction image will be applied to exported images if the correction image is available.

5.20.2 Export Region Overview

You can access this option from**:**

- **Region viewer → Export → Region Overview**;
- **Experiment Editor → Region Context Menu**.

- If you want to export more region overviews simultaneously, you can select multiple items from the Experiment Editor tree.

The following dialog will appear:

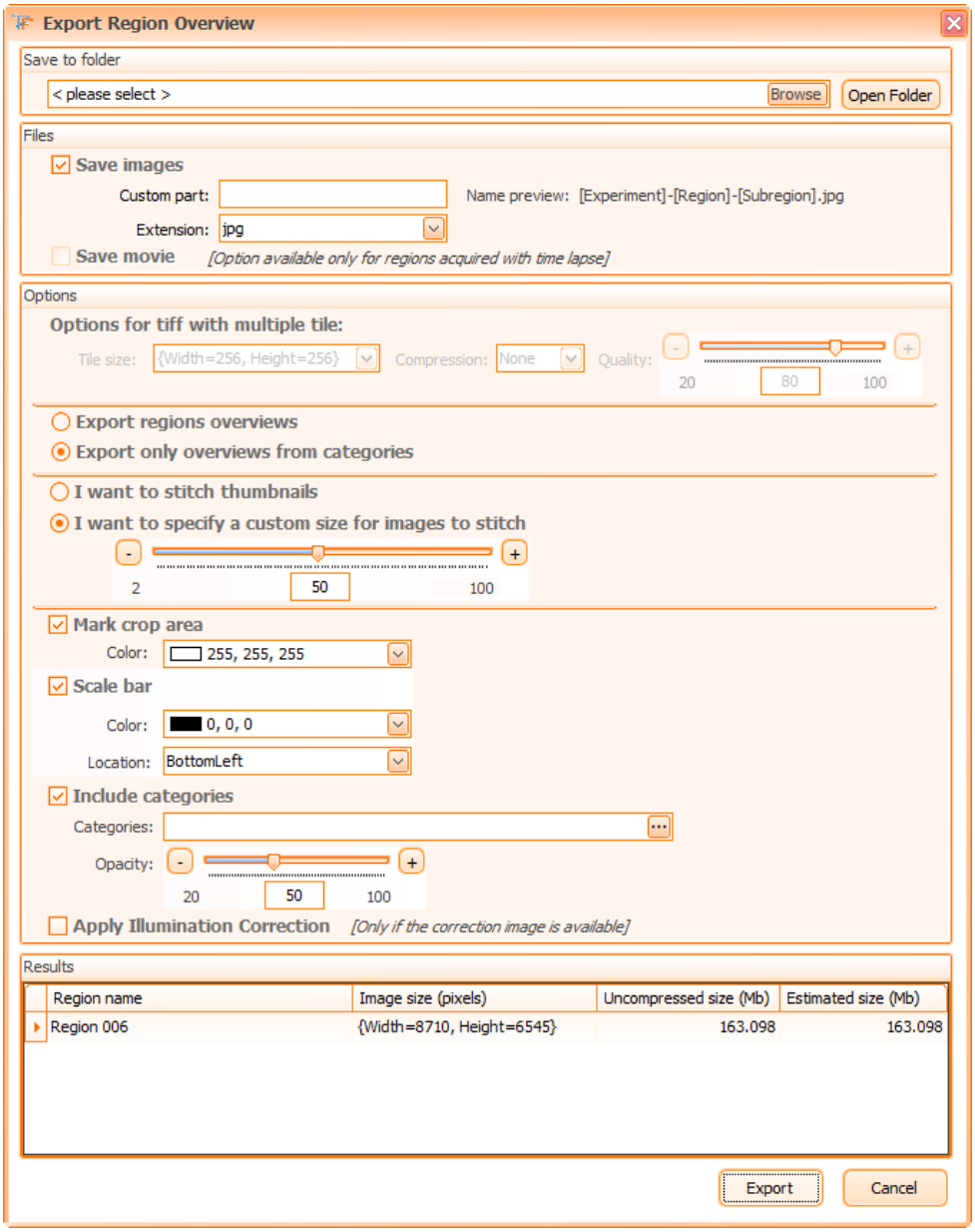


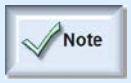
Figure 233 - Export Region Overview dialog

Some information is displayed for each region: **name, size, uncompressed size, estimated size**. If one of the regions is too large, the respective region will be highlighted in **red**.

TissueFAXS allows configuring the export as desired, by choosing exactly the contents of interests. In the dialog displayed above, the user can adjust the following **settings**:

- Select the **storage folder**;
- Select **files** to be exported:
  - Images
  - Movies (option available only if the region was acquired with time lapse)

- Enter **Custom Part**: type desired custom part for the name of the exported item.



- The file name to be exported consists of a default part and a custom part. The default part consists of items from the File Name Convention (see **Chapter 4.2.3**). The custom part is defined by the user.

- Select the file **extension**: choose exported item format (also available: *.tiff with multiple tiles* in order to export bigger images);

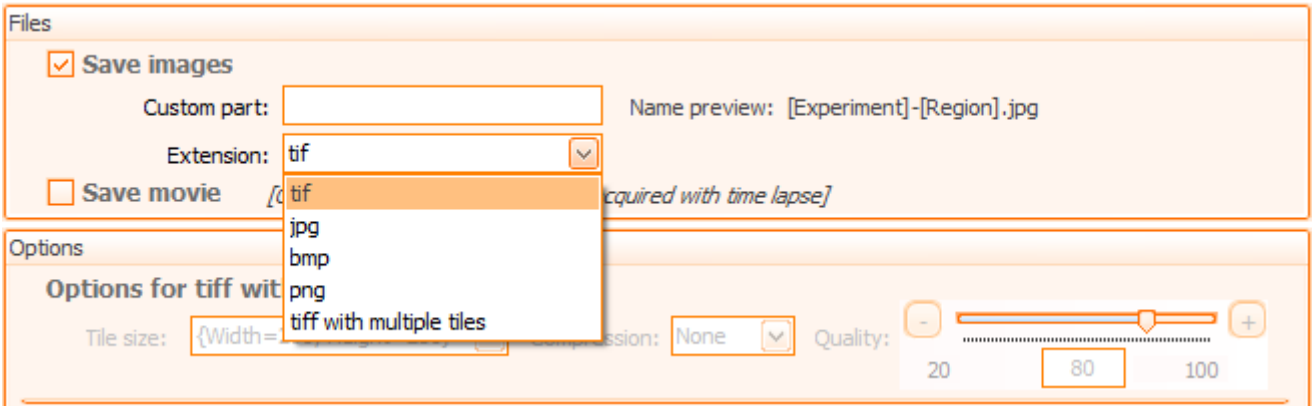
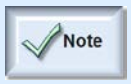


Figure 234 - Export Region Overview: Extensions

If choosing as extension the *tiff with multiple tiles*, the items from the **Options for tiff with multiple files** will be enabled:

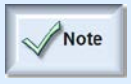
- **Tile size**: the dimension of an image (tile) composing the tiff (there are three predefined options);
- **Compression**: there are two options - **None** and ***jpeg***.
- **Quality**: the quality of the compression can be adjusted using the slider.



- If choosing as extension the *tiff with multiple tiles*, the following options will not be available anymore: Mark Crop Area, Scale bar, Include Categories.

- **I want to stitch thumbnails**: choose this to export a low resolution of the region;
- **I want to specify a custom size for images to stitch**: choose the desired size of the resulting export image. Be aware that, for larger images, there is a memory restriction that comes from the operating system and hence, this operation may not work if a system is low of memory;
- **Mark Crop Area**: it marks the contour of the region on the final image; you can select its color;
- **Scale bar**: please see **Chapter 4.1.2** of the current manual;
- **Include Categories**: in this case, the selected categories will appear on the final image.

Here the user can choose the categories to be included in the region overview image, by pressing the browse button ( ).



- In the selection list, only those categories will be listed that contain regions on their overview image.

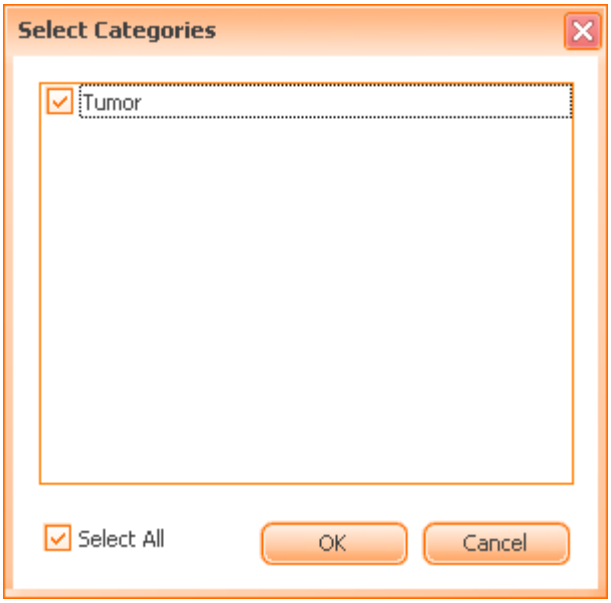


Figure 235 - Export Region Overview: Categories included

- **Opacity** of the categories: adjust the **Opacity** parameter, in order to choose the transparency of the tissue area name that appears on the region.
- **Apply Illumination Correction**: this option is enabled only for **Brightfield** experiments. The correction image will be applied to exported images if the correction image is available.

Note

Restrictions regarding the images to be exported:

- For all export formats (excluding *tiff with multiple tiles*) the image size must not exceed 80000000 pixels.
- For *tiff with multiple tiles* the uncompressed size must not exceed 2000 Mb.

Export Overviews from Categories

To export categories, first check **Export only overviews from categories**. You should also select the types of categories to be exported, by pressing the button. The following dialog will appear, listing the available categories:

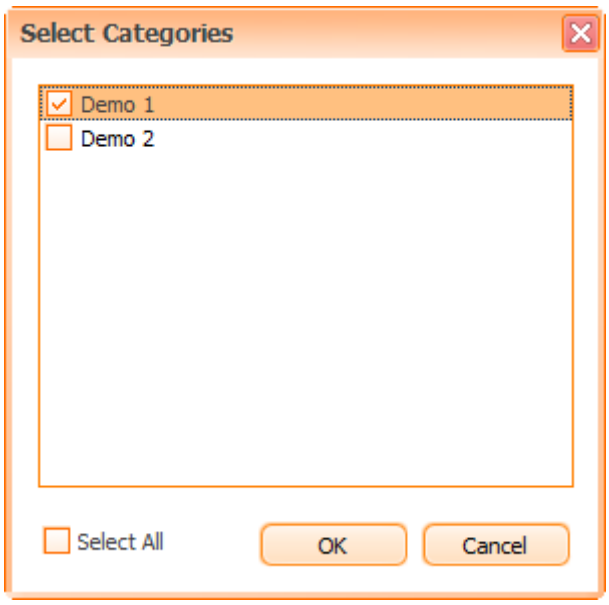


Figure 236 - Export subregions: Select categories

In the lower part of the **Export Region Overview** dialog you will now see a section including all the overviews that will be exported. For each overview the name and the image size is displayed.

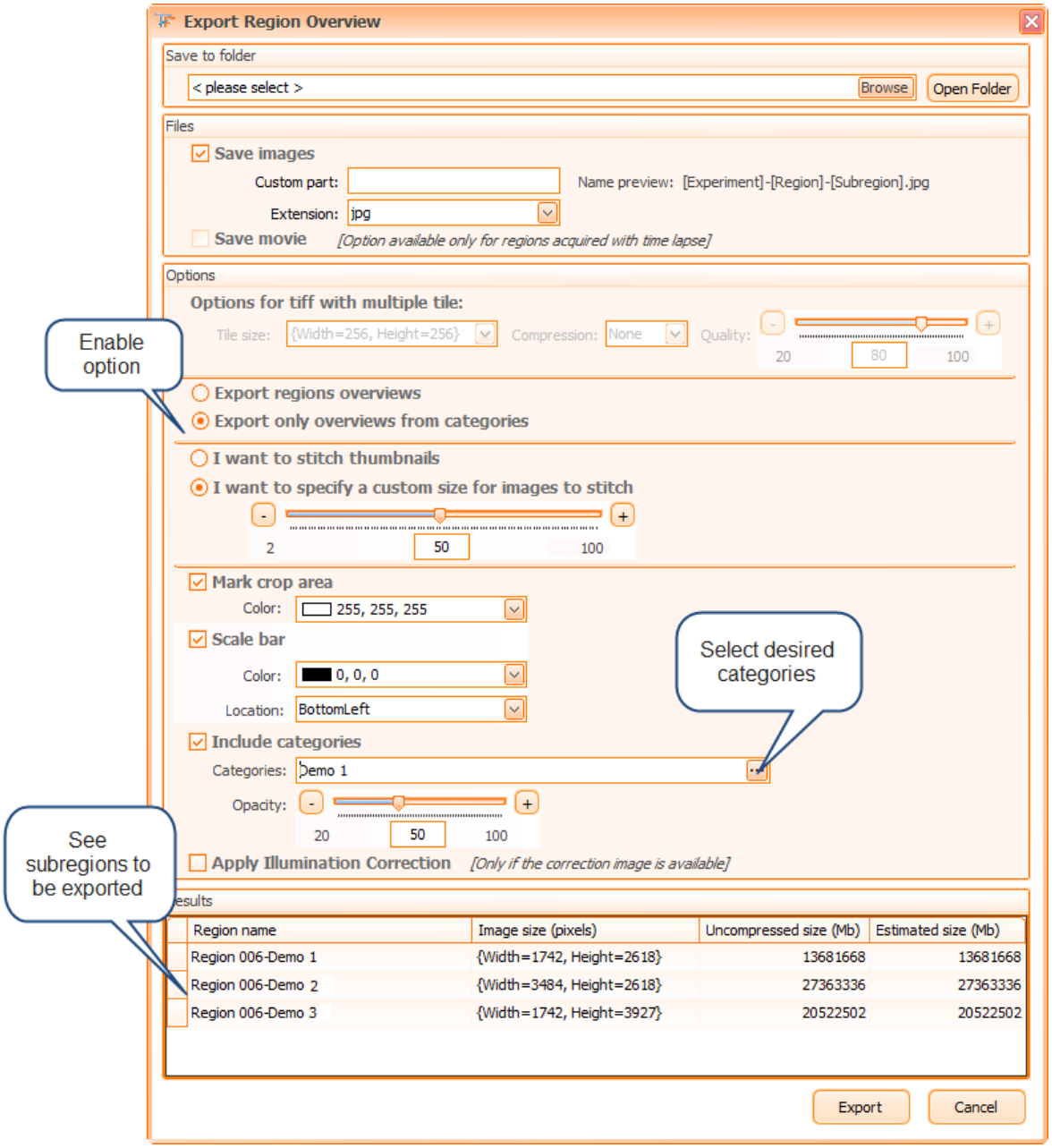


Figure 237 - Export Region Overview: export subregions

5.20.3 Export One FOV Image

You can access this option by pressing the **Export** button from the One Image Viewer toolbar.

Figure 238 - One Image Viewer toolbar

The following dialog will appear:

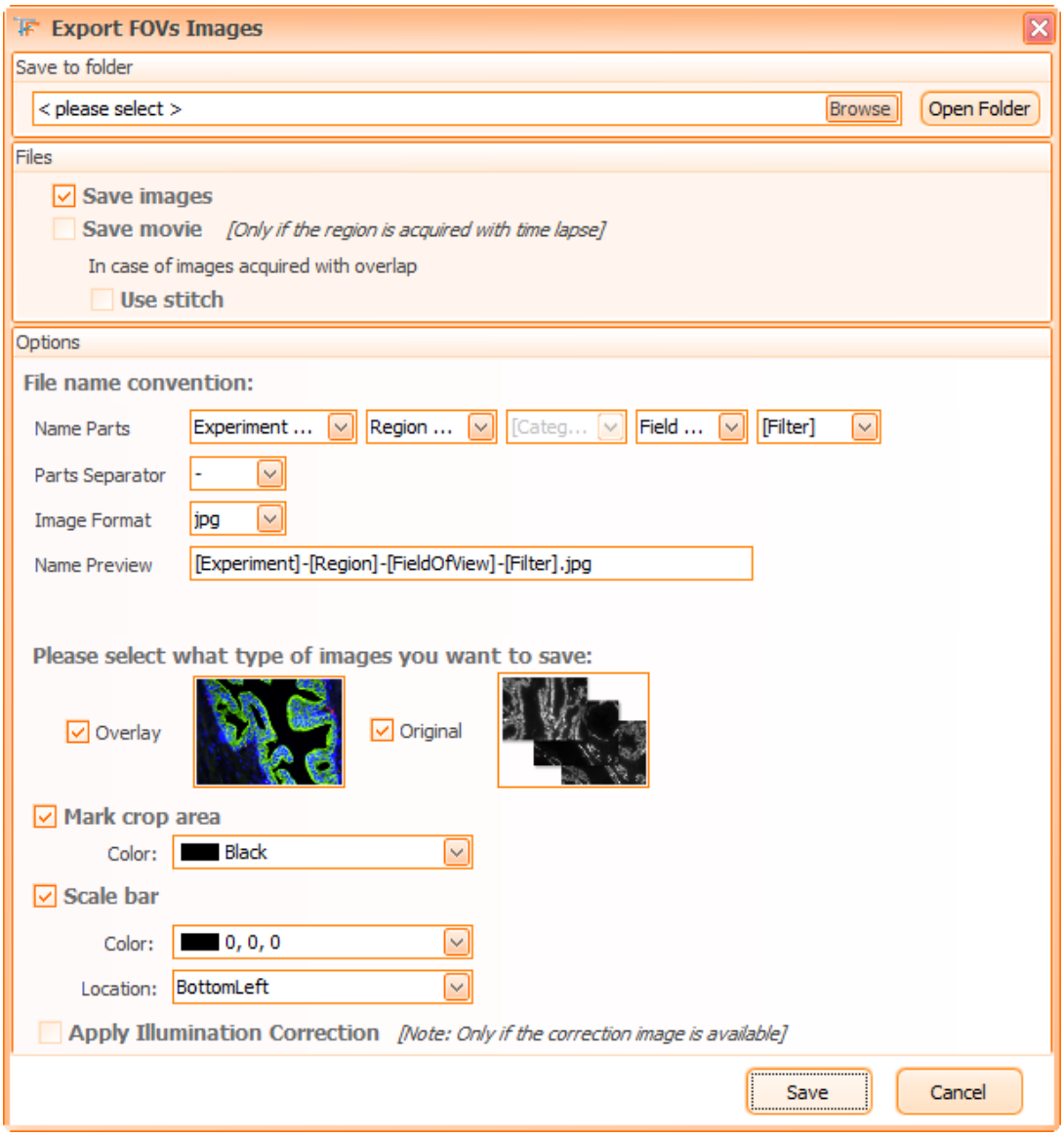


Figure 239 - Export single FOV Image dialog

- Select the **storage folder**;
- Select **files** to be exported:
  - Images
  - Movies (option available only if the region was acquired with time lapse)
  - Use stitch
- Select the **file name convention** (Name Parts, Custom Part, Parts, Image Format, Name Preview);
- Select the **type of images** you want to save:
  - **Overlay**: the image as it appears in the One FOV Viewer.
  - **Original**: the images for each channel as acquired.
- **Mark Crop Area**: it marks the contour of the shape for each FOV; you can select its color;
- **Scale bar**: please see **Chapter 4.1.2** of the current manual;
- **Apply Illumination Correction**: this option is enabled only for **Brightfield** experiments. The correction image will be applied to exported images if the correction image is available.

5.20.4 Export current Slide Image

To export a slide's preview image, select **Export slides images…** from:

- Stage control menu

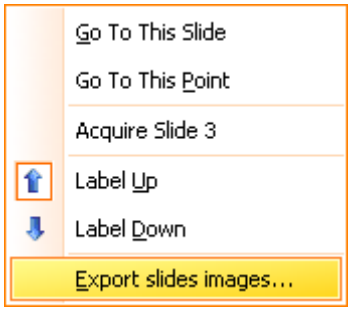


Figure 240 - Stage control context menu

- Slide editor menu

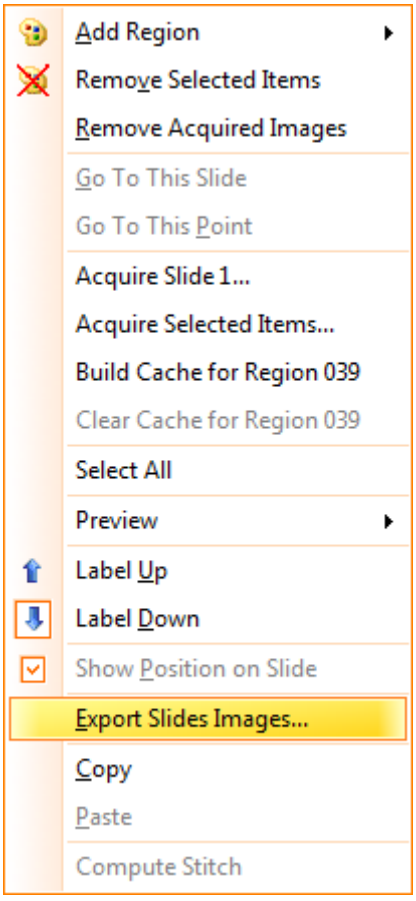


Figure 241 - Slide editor context menu

- Experiment editor menu only on experiment item

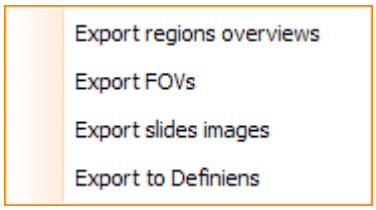


Figure 242 - Export slides images from Experiment editor

No matter from where you access this option, the following dialog will appear:

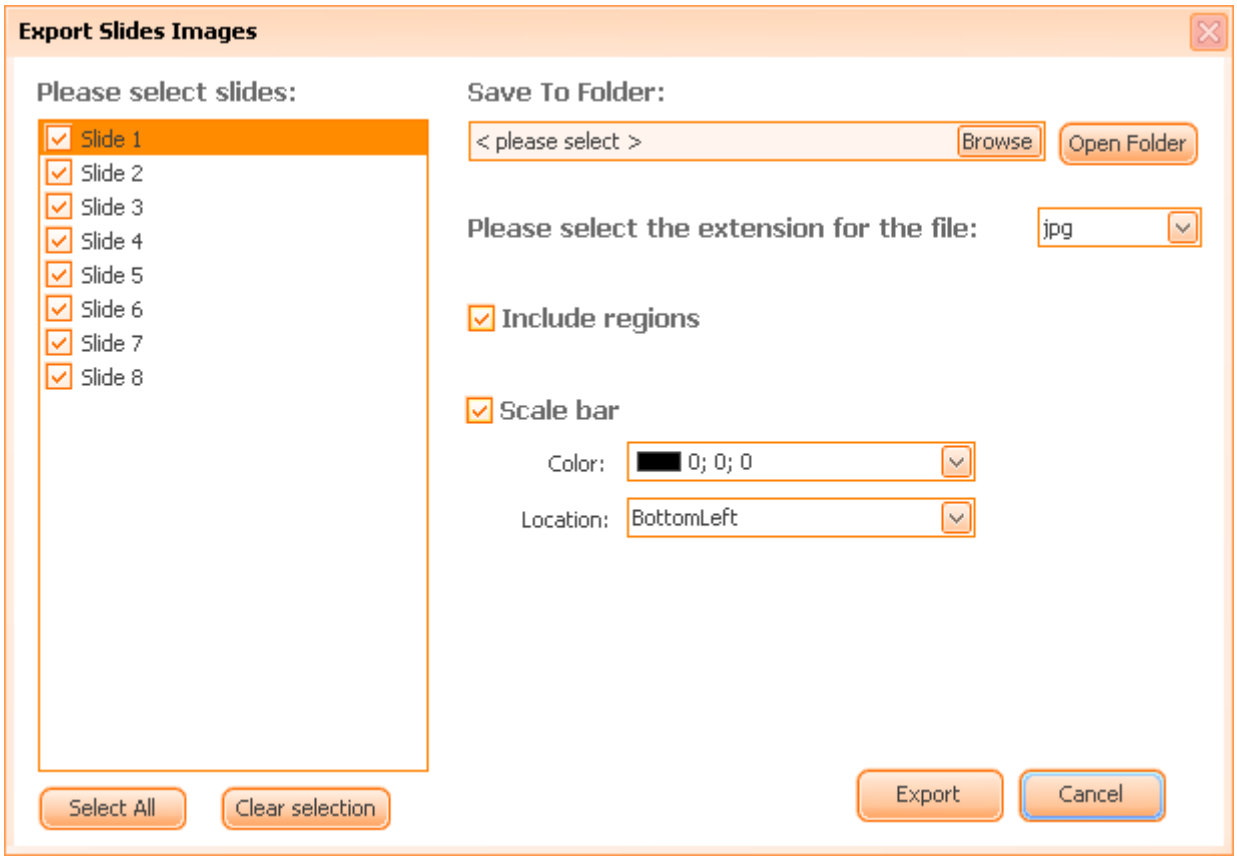


Figure 243 - Export this Slide Image dialog

- Select the storage folder;
- Select the file extension;
- **Include regions** checkbox - you can choose if the export image should contain or not the region shapes;
- **Scale bar**: please see **Chapter 4.1.2** of the current manual.

5.20.5 Export Annotations

To export annotations, go to the region viewer toolbar, choose **Subregions** button → **Export Annotations**.

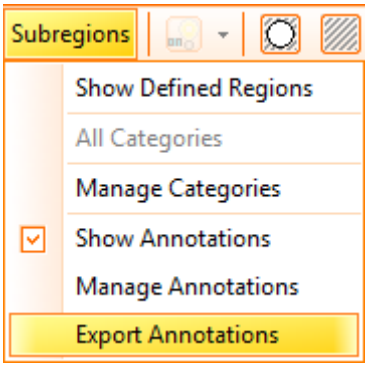


Figure 244 - Subregions contextual menu: Export Annotations

The following dialog will appear:

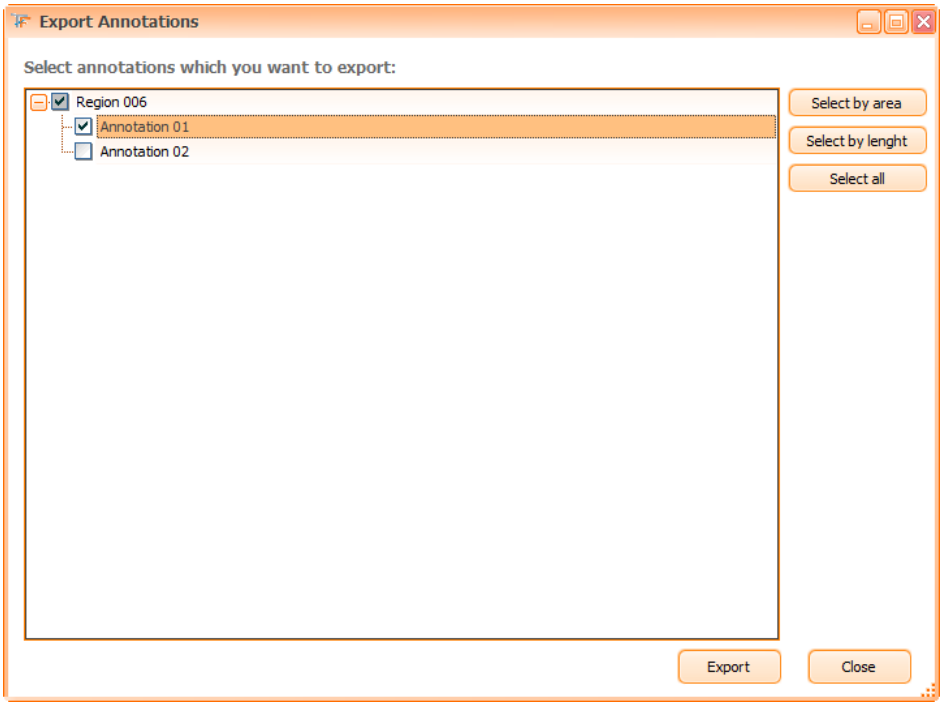


Figure 245 - Export Annotations

The first step to export is to select desired item(s) from the list.

You can also select desired items by using the buttons in the right side of the dialog:

- Select by area
- Select by length
- Select all

Now press **Export** button and choose in the browser that opens the location folder and specify the name for the *csv* file to be exported. Finally, press **Save** button to finish the export.

The exported *csv* file will contain the following data:

- Experiment name
- Slide name
- Region name
- Annotation name
- Area/Length

- Unit measure
- Description (if any)

5.21. Use Plugins

A plugin is a software program that extends another program's functionality without the necessity of reinstalling the main program. For **TissueFAXS**, the following plugins are provided by default:

Plugins for Brightfield experiments

- Launch **HistoQuest** for entire experiment from **Analysis → Launch HistoQuest…**;
- Launch **HistoQuest** for the current region displayed in region viewer from **Plugins → Analysis → Launch HistoQuest…**, in region viewer.

In both cases above, all version of **HistoQuest** (starting with **HistoQuest 1.0.2.0210**) will automatically import corresponding data from **TissueFAXS**.

Plugins for Fluorescence experiments

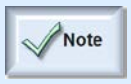
- Launch **TissueQuest** from **Analysis → Launch TissueQuest…**;
- Launch **TissueQuest** from **Plugins → Analysis → Launch TissueQuest…** in region viewer.

In both cases above, **TissueQuest** will start.

6. TMA Support

Although the TMAs can be treated and acquired as regular regions, **TissueFAXS** offers enhanced support for this type of samples/slides, in order to facilitate your work and obtain high quality results.

In order to define TMA blocks and spots on a slide, the content type of the slide must be set to **TMA**.



- It is not possible to set the content type of a slide if the slide contains any regions or groups.

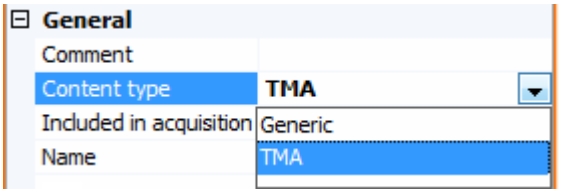


Figure 246 - Setting the content type of the slide

6.1. Automatic detection of TMA structures

TissueFAXS provides automated tissue detection for TMA structures. By using this feature, the efforts of working with TMAs will be considerably reduced.

It consists in an algorithm based on contrast and uniform illumination. Therefore, applying the illumination correction before detection of the TMA spots might be needed. You can do this by using **Illumination Correction** button ( ) from **Slide Preview** toolbar.

After applying the illumination correction, **TissueFAXS** can automatically detect the TMA structures on a slide.

Start by pressing the **Tissue Detection** button ( ) from **Slide Preview** toolbar.

When the automatic detection of TMA structures is initiated, the dialog below will appear. In this dialog, the user can restart the TMA detection using different parameters. This operation can be done for the whole slide or over a specific selected area. For best results, the area selected for detection should contain mostly tissue. The coverslip borders and other artifact structures should be avoided.

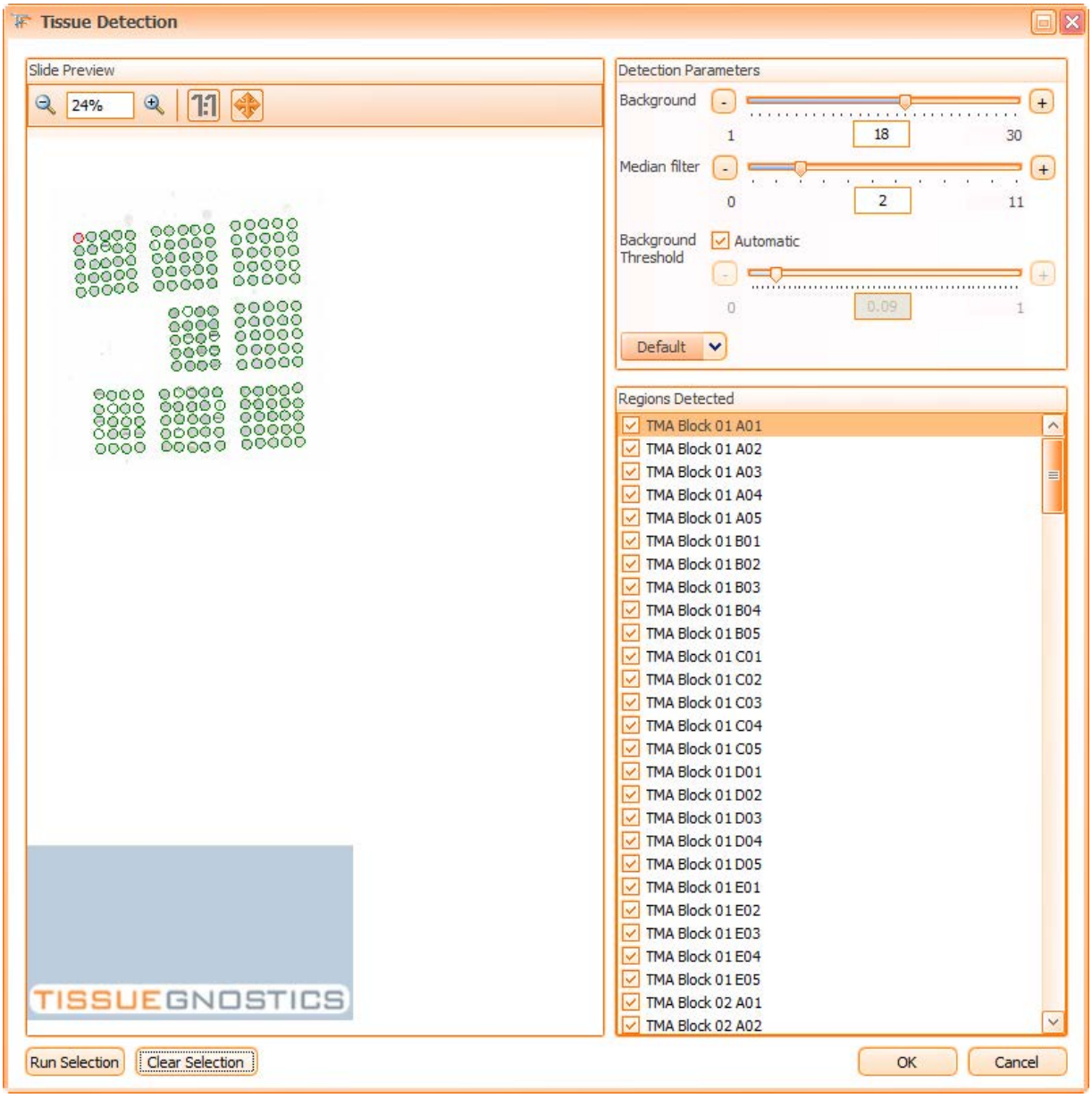


Figure 247 - Automatic detection of TMA structures panel

Parameters

In order to refine the results of the automatic detection of TMA structures, **TissueFAXS** provides a set of parameters that can be adjusted according to your needs, in order to obtain the desired results.

Background: Use this to eliminate the high background and ununiform illumination. Its value should be equal to the average size of the spot radius.

Median filter: Use this to improve detection when images containing some artifacts have to be used for detection. Basically, the algorithm will ignore the pixels brighter/darker than the medium value.

Background threshold: It is best to set this to auto. You might want to try this when the spots are clearly visible on the background and the detection fails to return the best results. This parameter represents a medium value between background maximum intensity and spots minimum intensity.

When TMA structures are adequately detected, press the **OK** button.

Place Holders

The **place holders** are virtual TMA spots which replace missing tissue from some positions on the matrix structure of the TMA block. In the image below, the **place holders** appear in *dark green*.

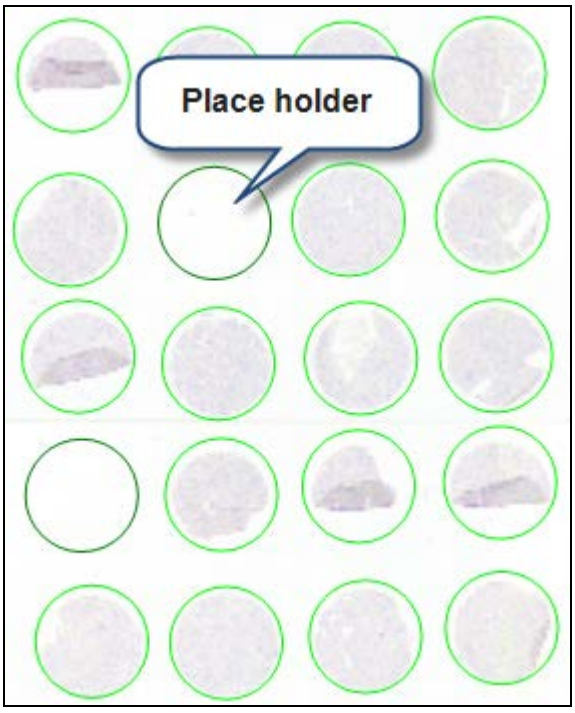


Figure 248 - Place holders

For a TMA spot to be considered a **place holder**, go to the property editor and set the value **Yes** in the **Placeholder** option.

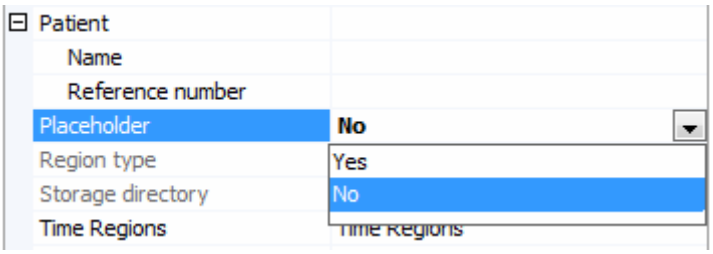


Figure 249 - Place holder property

Note

- The automatically detected empty spots already have this property set to **True** value.
- The **place holders** are not included in the acquisition process.

6.2. Manual definition of TMA structures

In order to manually define the TMA structures on a slide, the slide must already have a preview image and the content type of the slide must be set to **TMA**.

6.2.1 Definition of TMA block size

The first step in the manual definition of TMA structures is to indicate the size of a TMA block (the number of rows and columns).

You have two possibilities when defining the size of a TMA block.

- Press the left mouse button on **TMA Block Size** button ( ) and drag the mouse down and right while holding the mouse button until the desired size of the TMA block is defined.

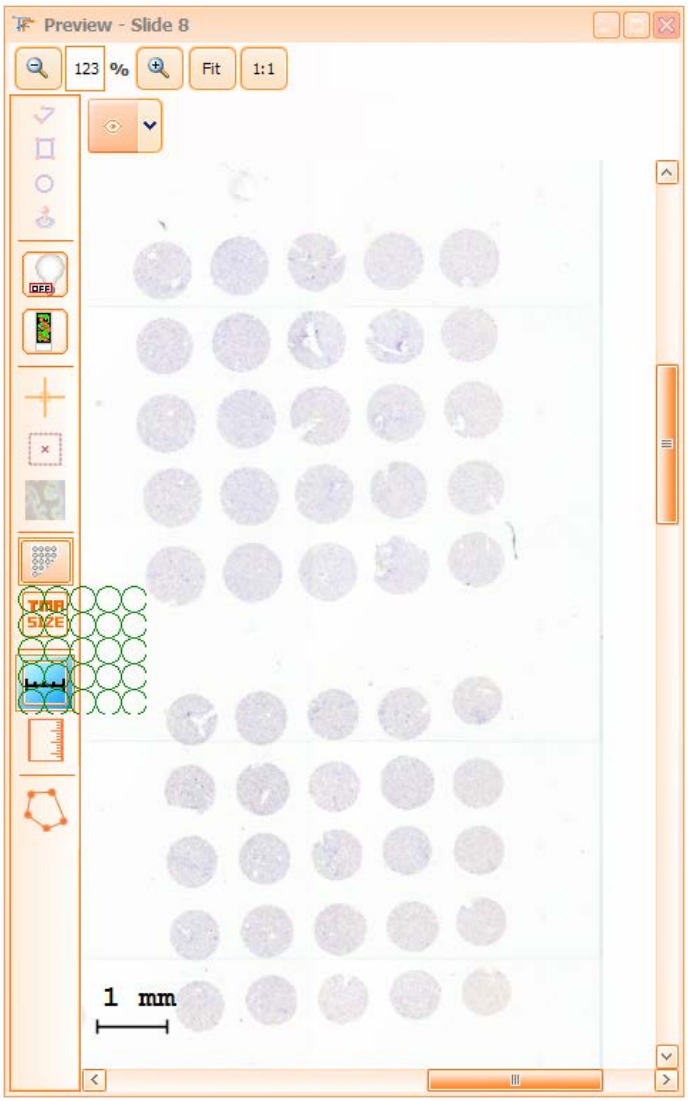


Figure 250 - Definition of the TMA block size

- Using predefined size for the TMA blocks, by pressing **TMA size** button ( ). A dialog will appear where the desired number of rows and columns can be set.

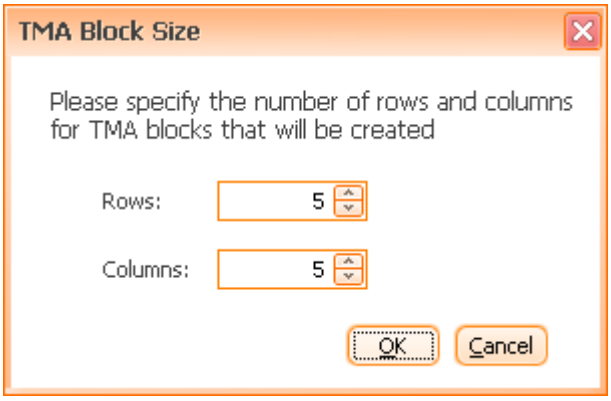


Figure 251 - TMA block size dialog

6.2.2 Definiton of the first TMA block on slide

The first TMA block on a slide is defined by indicating the diagonal between the most upper-left and the most lower-right TMA spots of the TMA block.

To indicate this diagonal, follow the steps below:

- hold the **SHIFT** key;
- press the left-mouse button in the center of the upper-left TMA spot of the TMA block;
- holding the left-mouse button, drag a diagonal to the center of the lower-right TMA spot in the TMA block;
- when you finish drawing the diagonal, release the left-mouse button and then release the **SHIFT** key.

This TMA block will become the template for all the other TMA blocks having the same size (the same number of rows and columns).

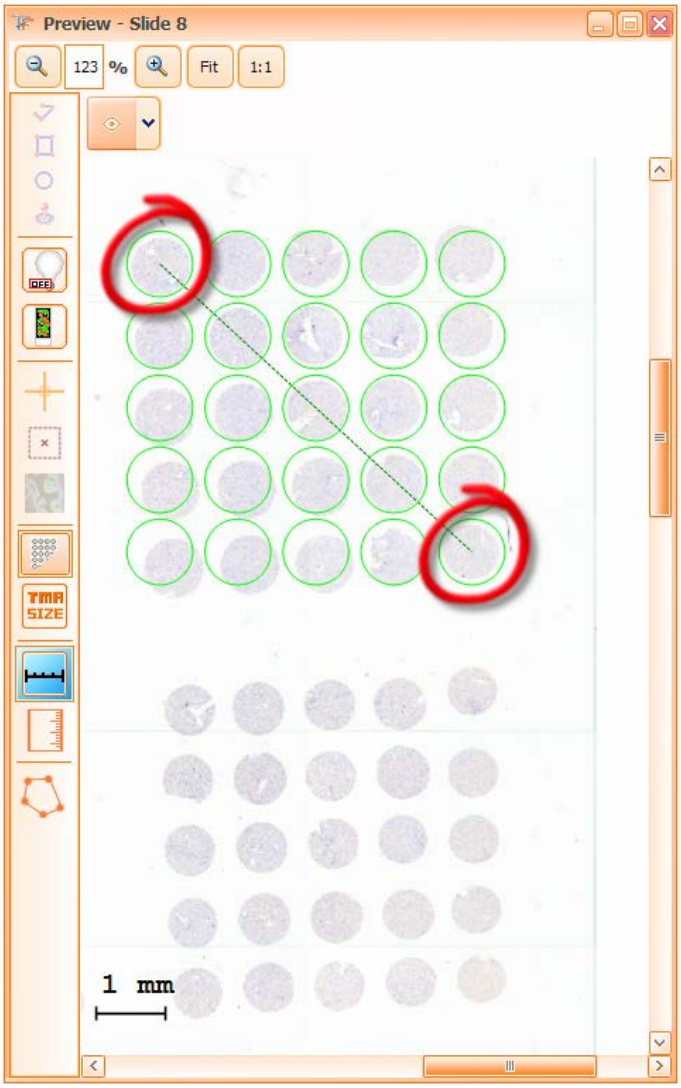


Figure 252 - Manual definition of the first TMA block on the slide

6.2.3 Definition of the second TMA block on slide

The second TMA block on slide is defined by indicating the center of the TMA spot in the most upper-left corner of the TMA block.

- hold the **SHIFT** key;
- click the left-mouse button in the center of the upper-left corner of the TMA block;
- release the **SHIFT** key.

The second and subsequent TMA blocks will have the same size as the last TMA block manually added using the first method.

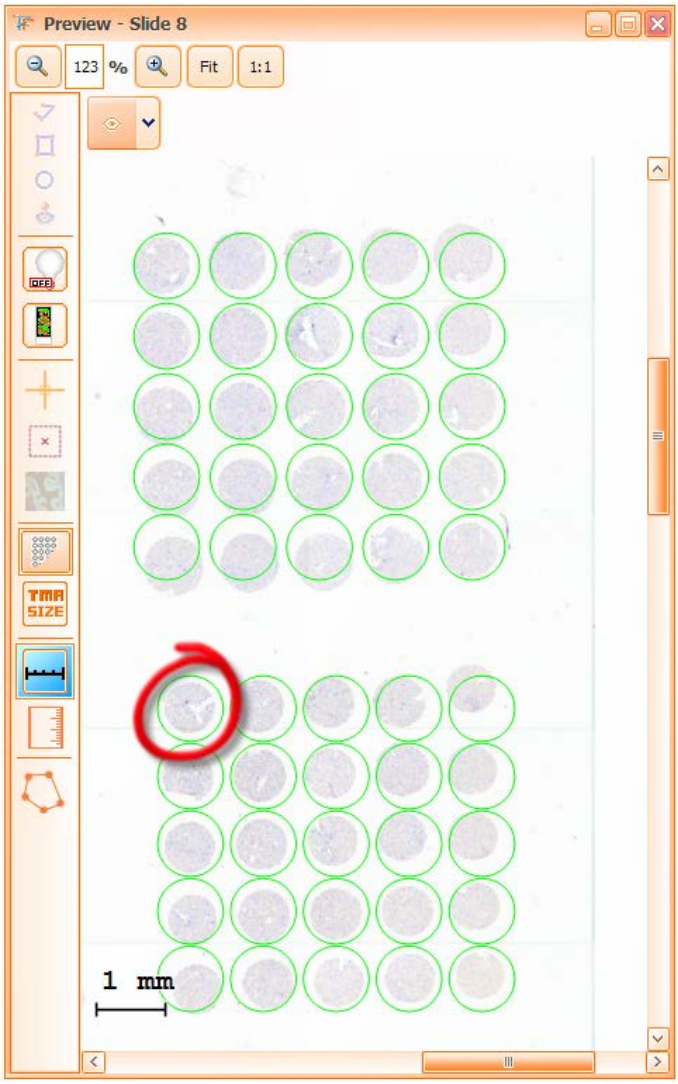


Figure 253 - Manual definition of the second TMA block on the slide

6.2.4 Selection of TMA blocks and spots

The TMA blocks and spots can be selected as below:

- select an individual TMA spot by clicking on it with the left-mouse button;
- select an individual TMA block by clicking in the free area between the TMA spots with the left-mouse button;
- select portions of the TMA block by drawing a rectangle that surrounds the desired TMA spots and blocks;
- select multiple TMA spots, by holding **CTRL** key;
- select the entire content of the slide by pressing the **CTRL**+ **A** keys.

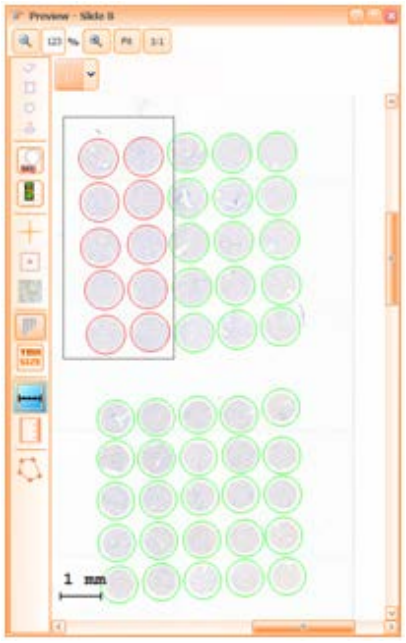


Figure 254 - Multiple selections with rectangle

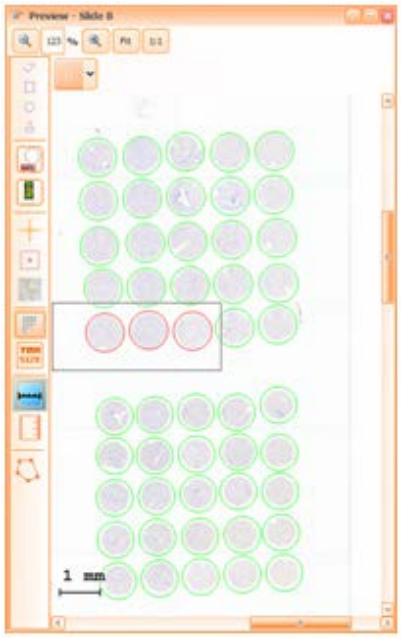


Figure 255 - Multiple selections with CTRL

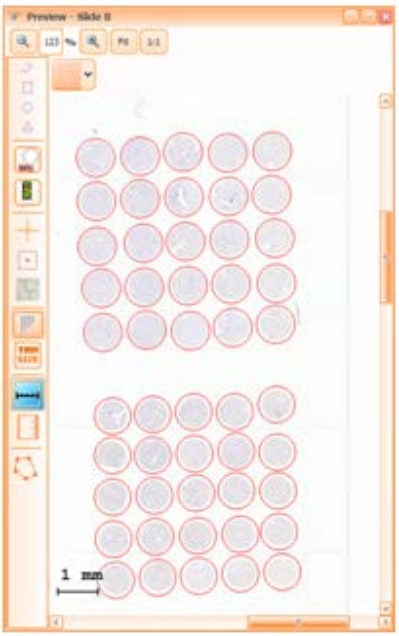


Figure 256 - Select all

6.2.5 Resize the TMA spots

The TMA spots can be resized as follows:

- select the desired TMA blocks or spots;
- use **CTRL**+ **+** keys to increase the size of the TMA spots;
- use **CTRL**+ **-** keys to decrease the size of the TMA spots.

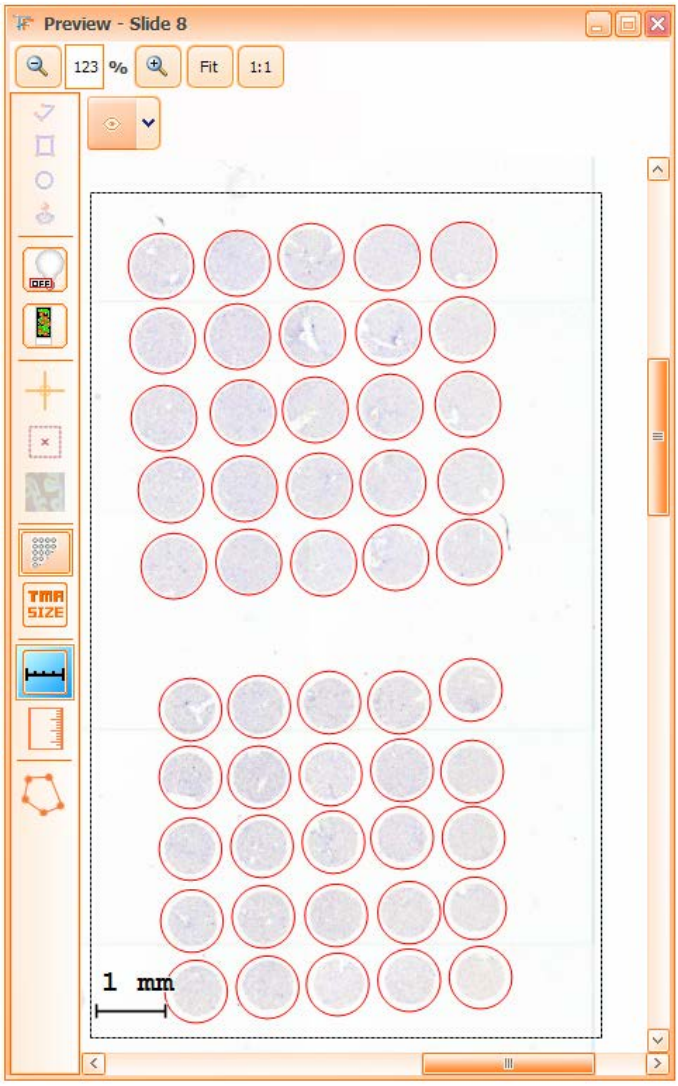


Figure 257 - Resize the TMA spots

6.2.6 Rotate a TMA block

The TMA blocks can be rotated as follows:

- place the mouse cursor next to the lower-right corner of the TMA block;
- press the left-mouse button on the blue arc that appears next to the lower-right corner of the TMA block and rotate the TMA block using the mouse;
- release the left-mouse button when finish.

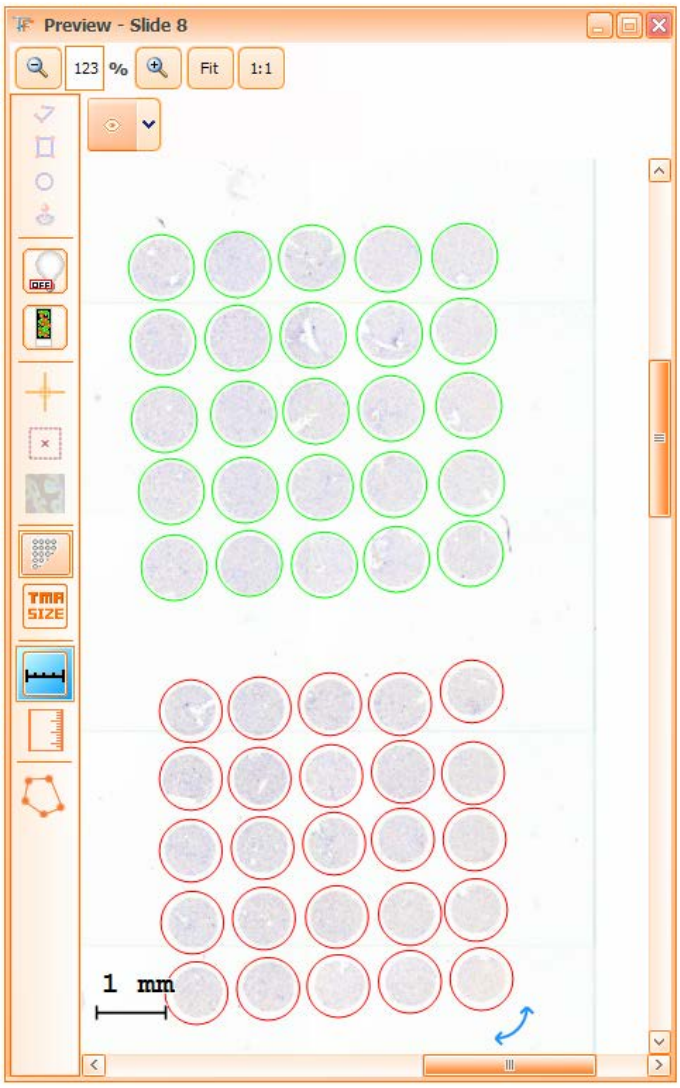


Figure 258 - Rotation of TMA blocks

6.2.7 Adjust the distance between TMA spots

The distance between TMA spots can be adjusted as follows:

- select the desired TMA spots;
- increase the distance on the X axis using **CTRL**+ → keys;
- decrease the distance on the X axis using **CTRL**+ ← keys;
- increase the distance on the Y axis using **CTRL**+ ↓ keys;
- decrease the distance on the Y axis using **CTRL** + ↑ keys;

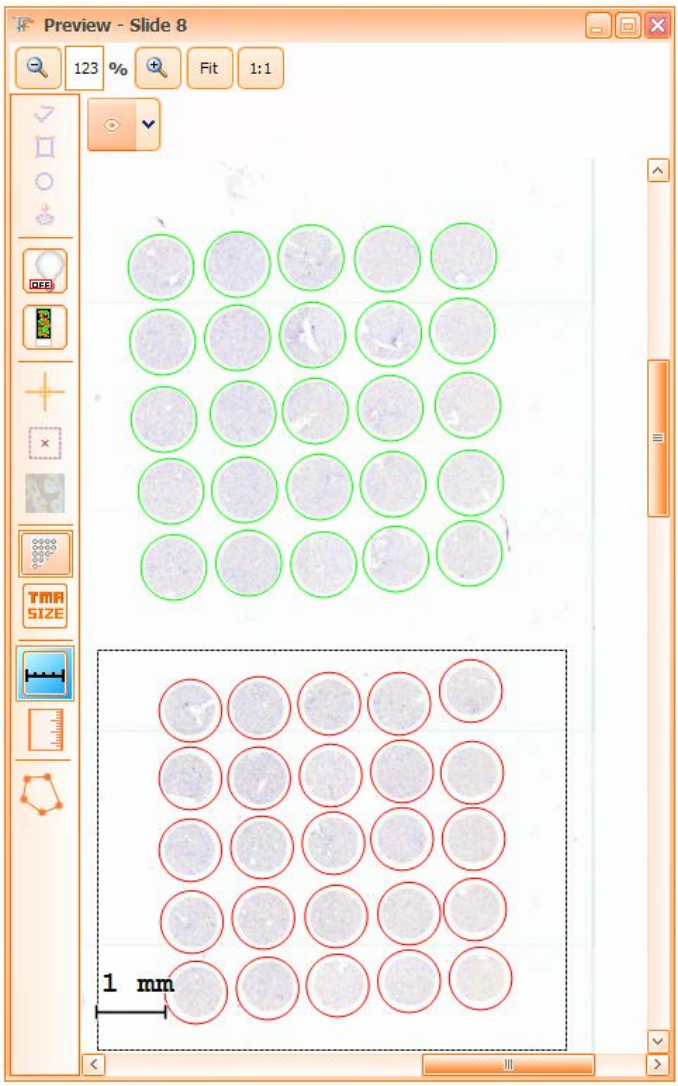


Figure 259 - Increase / Decrease the distance between TMA spots

6.2.8 Moving TMA blocks and spots

The TMA blocks and spots can be moved as below:

- select the desired TMA blocks and spots;
- move the selected objects using the mouse or ←,→,↑,↓ arrow keys.

6.2.9 Deleting TMA blocks and spots

The TMA blocks and spots can be deleted as follows:

- select the desired TMA blocks and spots;
- press the right-mouse button and select **Remove selected items** in the menu that appears, or press the **Delete** key.

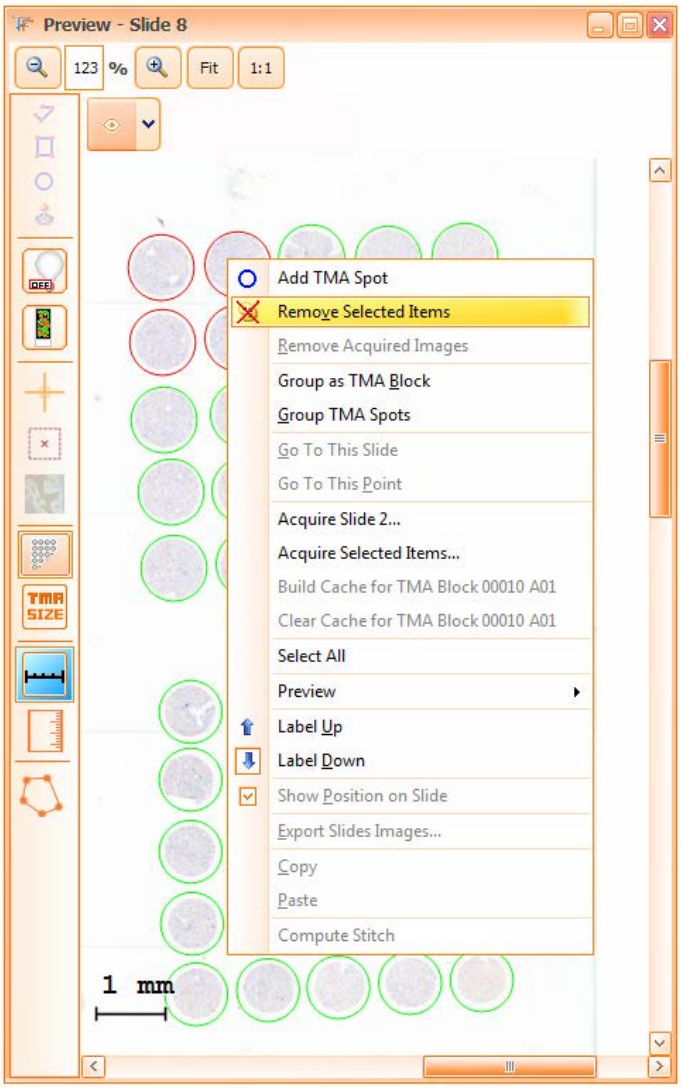


Figure 260 - Delete TMA spots

6.2.10 View acquired images for TMA spots

To view the acquired image of a TMA spot, double click on it.

6.2.11 Grouping TMA spots

When working with TMAs, it might become necessary to group certain spots within a block (they share similar properties, they contain certain types of cells etc.). So, TMA spots can be grouped as follows:

- select the desired TMA spots;
- press the right-mouse button and select **Group TA Spots** in the menu that appears.

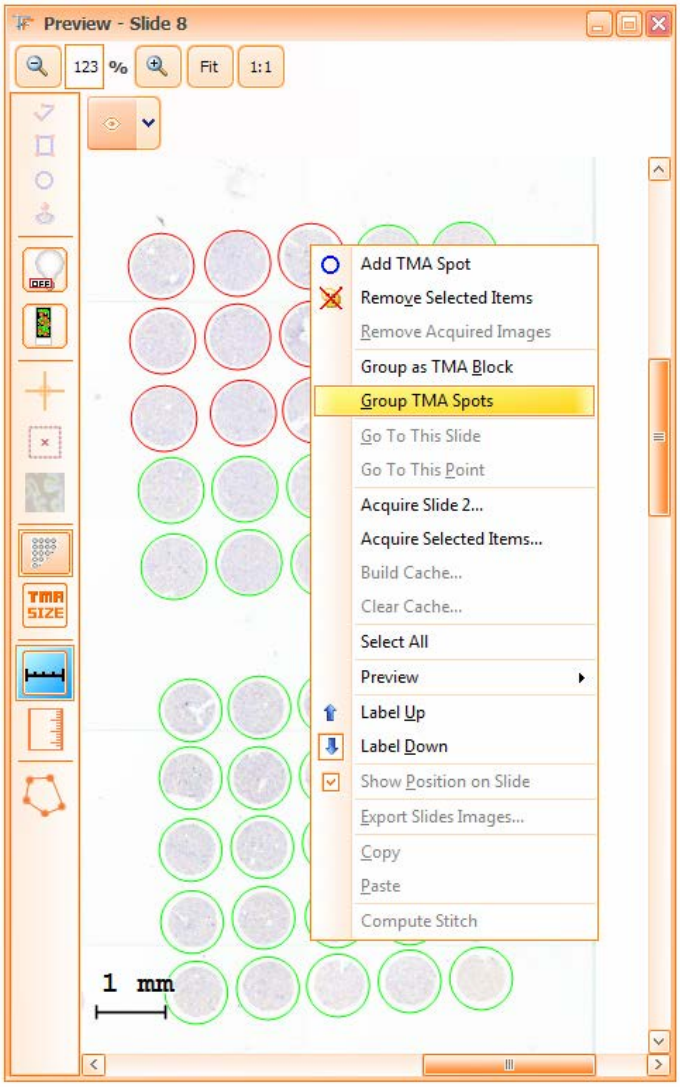


Figure 261 - Grouping TMA spots

Note

- **Time Lapse acquisition** mode is not available for TMA samples. Therefore, the **Acquisition Information** dialog that appears before acquiring TMAs will display a **Time Lapse acquisition** option disabled and a message that warns the user about this fact (see image below).

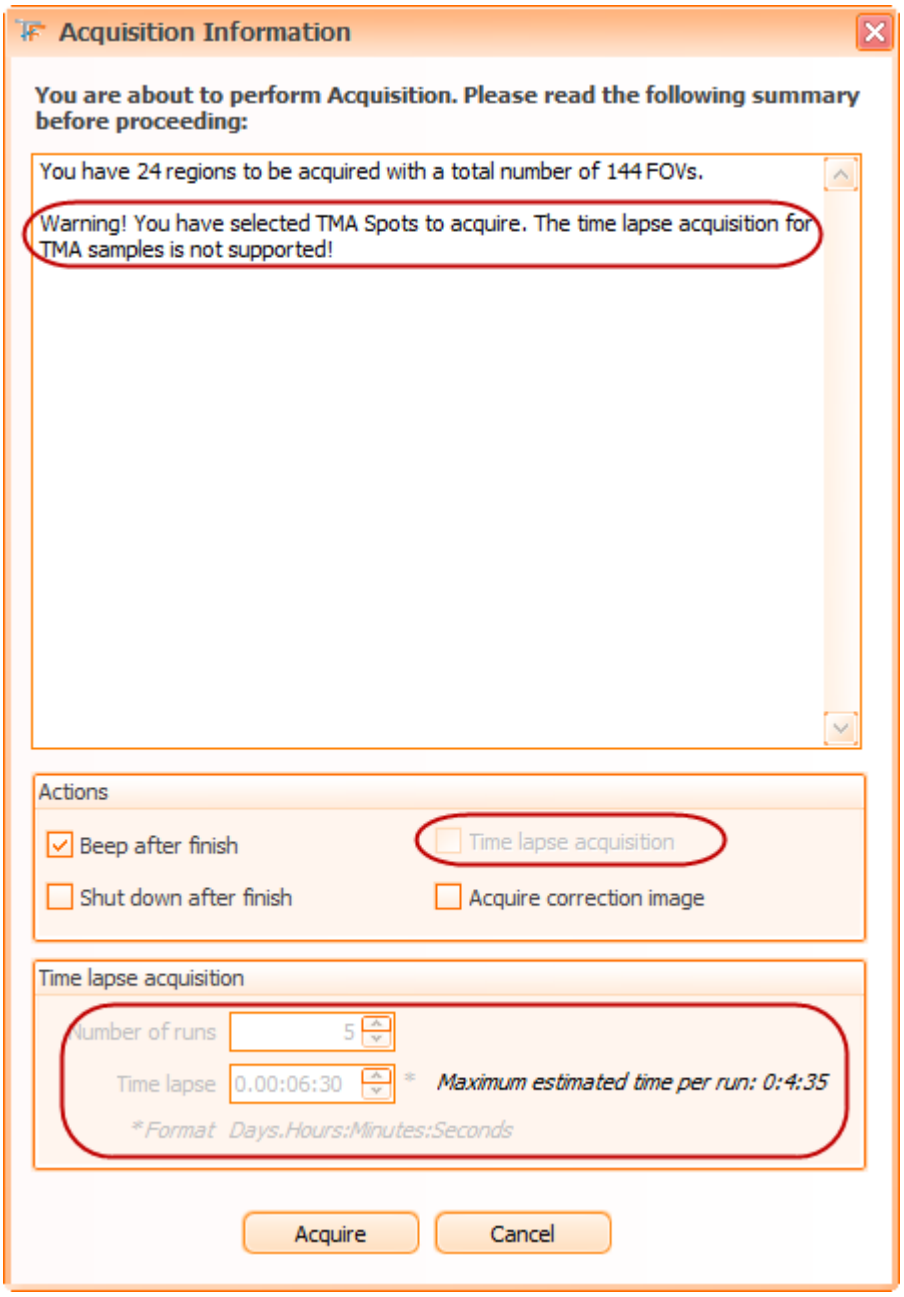


Figure 262 - Acquisition Information dialog: acquiring TMAs

7. Print

Printing your **TissueFAXS** project will prove to be very useful at some point, especially for the fact that the software will not simply print your project, but you will be able to decide which information the report has to contain and how the data will be displayed.

7.1. Print Options

You have two options to access the **Print** option and the **Print Preview** option:

- By pressing **Print** ( ) / **Print Preview** ( ) buttons from the main toolbar;
- By accessing **File** → **Print** or **File** → **Print Preview**.

Printing is only possible for an opened experiment. First, a dialog appears and then items to be printed must be selected (acquired items, all items).

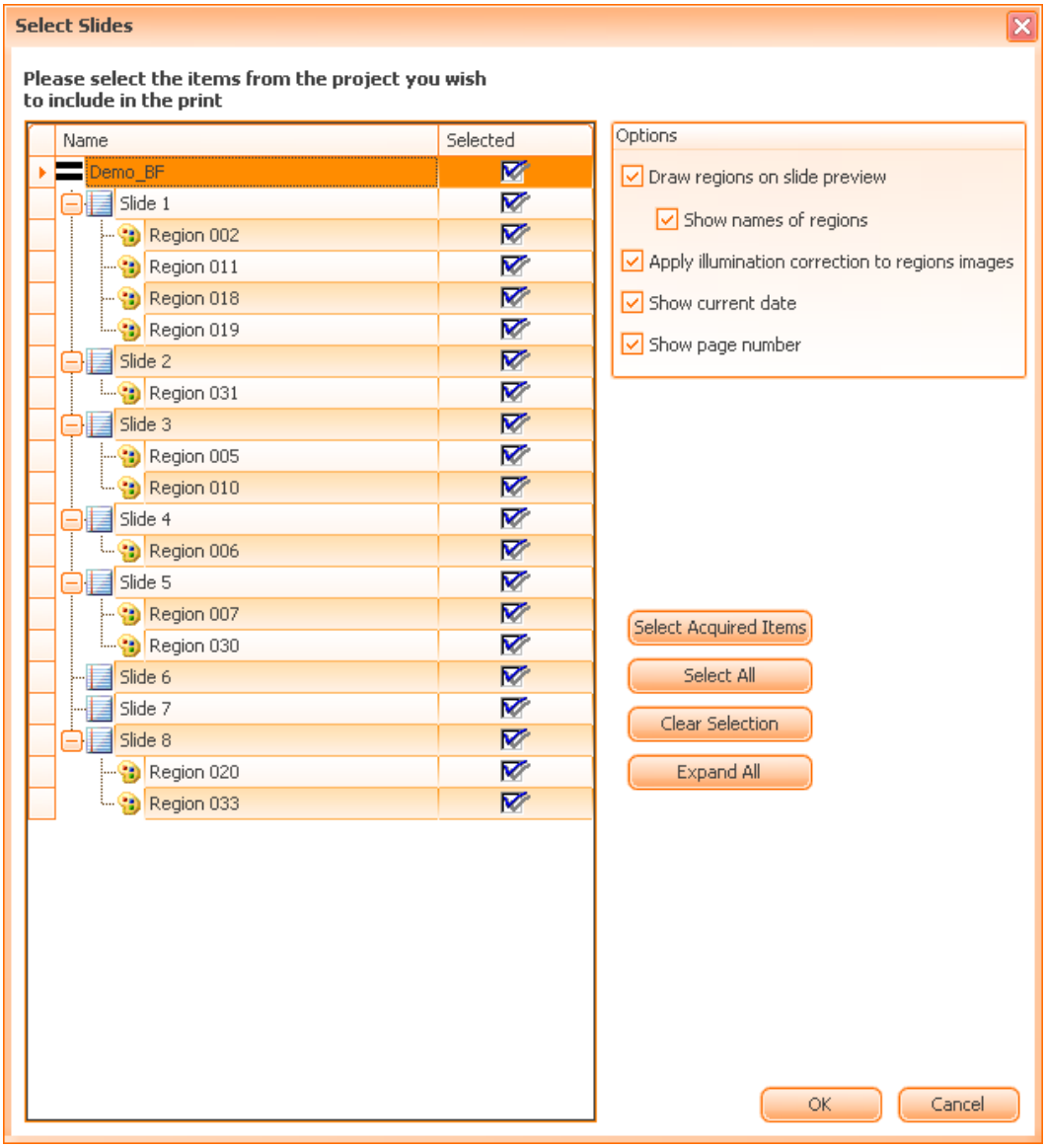


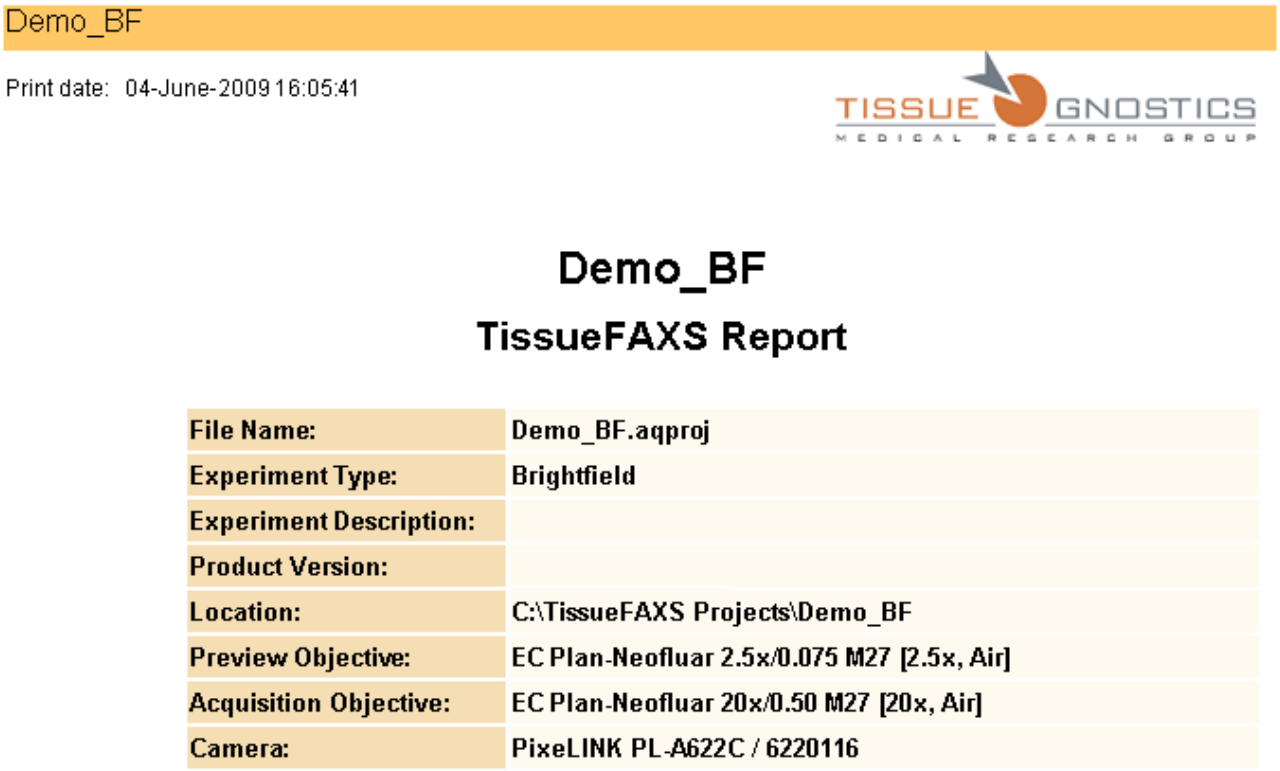
Figure 263 - Print dialog: choosing items to print

The default selection for this dialog is the selected slide in the slide viewer. It also offers a set of **options**:

- Draw regions on slide preview: the preview image may also contain region shapes;
- Show names of regions: this option is available only if the first option is selected;
- Apply illumination correction to regions: available only for **Brightfield** experiments, the correction image will be applied to exported images if the correction image is available;
- Show current date: the print date is visible on each page of the report;
- Show page number: the page number is visible on each page of the report.

7.2. Print Items

- If you choose the **Print** option, a dialog will appear that permits to choose the printer.
- If you choose the **Print Preview** option, you will be able to see a preview report.


Demo_BF

Print date: 04-June-2009 16:05:41

TISSUE GNOSTICS
MEDICAL RESEARCH GROUP

Demo_BF

TissueFAXS Report

File Name:	Demo_BF.aqproj
Experiment Type:	Brightfield
Experiment Description:	
Product Version:	
Location:	C:\TissueFAXS Projects\Demo_BF
Preview Objective:	EC Plan-Neofluar 2.5x/0.075 M27 [2.5x, Air]
Acquisition Objective:	EC Plan-Neofluar 20x/0.50 M27 [20x, Air]
Camera:	PixeLINK PL-A622C / 6220116

Figure 264 - Preview report example: first page

The **preview report** contains the following:

- Experiment Name;
- File Name;
- Experiment Type;
- Experiment Description;
- Product Version: the **TissueFAXS** version used to print this info;

- Location: the location of the experiment;
- Preview Objective: the objective lens used for the preview operation;
- Acquisition Objective: the objective lens used for acquisition;
- Camera: the camera used for this experiment;
- Each Slide selected in the list:
  - Slide Name;
  - Slide Image;
  - Content Type;
  - Comments: the comments referring to this slide;
  - Objective: the objective lens used for preview.
- Slide Preview Channels: a table that contains the channel list for the current slide and some properties for each channel in the list:
  - Checked: this flag indicates if the current channel is used for overlay;
  - Name: the channel name;
  - Intensity: the channel intensity;
  - Color: the channel color.
- Region list for each generic slide:
  - Region Name;
  - Region Image;
  - Comments: the comments referring to current region;
  - Acquired: this flag indicates if current region is acquired or not. Two possible values are present: **Yes** or **No**;
  - Path: the path for current region files;
  - Objective: the objective used for acquire current region;
  - Rows: the number of rows for region;
  - Columns: the number of columns for region;
  - FOV's Count: the number of FOVs items;
  - Patient Name: the patient name;
  - Patient Reference number: the individual reference number;
  - Time Lapse (if acquired with time lapse);
  - Number of Runs (if acquired with time lapse);
  - Time between Runs (if acquired with time lapse).
- Regions Channels: a table that contains the channels list for current region and some properties for each channel in the list:
  - Checked: this flag indicates if the current channel is used for overlay;
  - Name: the channel name;
  - Intensity: the channel intensity;
  - Color: the channel color.
- TMA Blocks list for each TMA slide:
  - TMA Block Name;
  - TMA Block Image;
  - Comments: the comments referring to current TMA block;

  - Acquired: this flag indicates if current TMA block is acquired or not. Two possible values are present: **Yes** or **No**;
  - Objective: the objective lens used for acquisition of the current region;
  - Rows: the number of rows for the current region;
  - Columns: the number of columns for the current region.
- TMA Spots Count: the number of spot items.

8. Help Menu

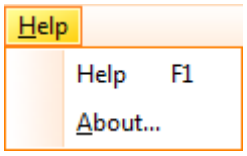


Figure 265 - Help menu

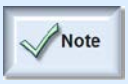
Help - press this button or F1 key in order to open the ***TissueFAXS User Manual***.

About... - this option will display a splash screen containing the main information about the **TissueFAXS** version in use and also the **Disclaimer** section (scroll to read).

Figure 266 - *About TissueFAXS* splash screen

9. Modularization

TissueFAXS application requires license for some of its features that you can find listed below.



- Please note that the behavior of **TissueFAXS** viewer is not depended on any dongle or any other licensing mechanism.

- Licenses that have a date time as expiry date will be checked each time the feature is used.

- Licenses that have a limited number of uses will be incremented as described in the table. In the vast majority of cases, the use counter will be incremented after an acquisition is done (with the feature enabled). When we are saying acquisition we are taking into account reacquisition also. When we are saying region we are referring to one of the following (based on context): *TMA Spot*, *Normal Region* and *Time Region*. So, if we acquire with stitch, same region for 20 times the stitch counter will be increased by 20.

Number of fluorescence channels

Projects created with a higher number of channels will be opened but acquisition will not be possible. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails. Checked only at experiment creation and acquisition.

Transmission in fluorescence experiments

Project created with Transmission in the list will not be acquired. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails. Checked at experiment creation/acquisition.

Acquisition of ZStack

TissueFAXS will not acquire Z stack. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Extended focus

TissueFAXS will not acquire using extended focus. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Acquisition of time-lapse regions

TissueFAXS will not acquire snapshots, but will open already acquired projects. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Acquisition with stitch and compute stitching

TissueFAXS will not acquire with stitch or recomputed stitch for regions. Older projects will be properly displayed. If the user owns a predefined number of uses, the counter will be incremented each time stitch is computed manually or automatically through the acquisition.

Acquisition of TMA

TissueFAXS will not acquire TMA Spots, but will allow opening existing projects. If the user owns a predefined number of uses, the counter will be incremented each time a TMA spot is acquired.

Acquisition of well plates

TissueFAXS will not acquire Well Plates, but will allow opening existing projects. If the user owns a predefined number of uses, the counter will be incremented each time a region on a Well Plate is acquired.

Tissue Detection

TissueFAXS will not allow running Tissue Detection. If the user owns a predefined number of uses, the counter will be incremented each time Tissue Detection is runned.

Illumination Correction

TissueFAXS will not allow acquiring/setting the illumination image. If the user owns a predefined number of uses, the counter will be incremented each time the correction images are applied.

Burst mode for Baumer during focus and acquisition

TissueFAXS will not allow the use of burst mode during acquisition/focus. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Correction flags

TissueFAXS will not allow the use for flags. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Load settings from file

TissueFAXS will not allow loading settings from project. If the user owns a predefined number of uses, the counter will be incremented after each load.

Load settings from profile

TissueFAXS will not allow loading settings from profile. If the user owns a predefined number of uses, the counter will be incremented after each load.

Camera profiles

TissueFAXS will not allow loading settings from profile. If the user owns a predefined number of uses, the counter will be incremented after each load.

Build cache for acquired regions

If license is not present, TissueFAXS will not display/compute the cache and it will use thumbnails only. If the user owns a predefined number of uses, the counter will be incremented after each build.

Export FOV images

TissueFAXS will deny export if license is not present. If the user owns a predefined number of uses, the counter will be incremented after each region export.

Export Region Overviews

TissueFAXS will deny export if license is not present. If the user owns a predefined number of uses, the counter will be incremented after each region export.

Export movie for time-lapse

TissueFAXS will deny export if license is not present. If the user owns a predefined number of uses, the counter will be incremented after each region export.

Export of XML files

TissueFAXS will deny use of plug-in based on dongle. If the user owns a predefined number of uses, the counter will be incremented after each export.

Import regions from XML files

TissueFAXS will deny use of plug-in based on dongle. If the user owns a predefined number of uses, the counter will be incremented after each export.

Export to Definiens

TissueFAXS will deny use of plug-in based on dongle. If the user owns a predefined number of uses, the counter will be incremented after each export.

Acquire raw data images

TissueFAXS will not save raw data during acquisition. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Measure Tool

TissueFAXS will not allow the use of measure tool. If the user owns a predefined number of uses, the counter will be incremented after each use.

Print Module

TissueFAXS will not allow the use of print module. If the user owns a predefined number of uses, the counter will be incremented after each use.

Role-based administration

No settings will be hidden based on user role. If the user owns a predefined number of uses, the counter will be incremented after each login.

Estimate and display duration

No estimation will be displayed. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Move the Z axis on filter change

Z drive will not move when changing filters. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will not be incremented if acquisition fails.

Different Attenuator settings for channels

Attenuators settings will be ignored when moving from filter to filter. TL Lamp settings will be ignored. If the user owns a predefined number of uses, the counter will be incremented after each successful or canceled acquisition. The counter will be incremented if acquisition fails.

If the user does not have a license for a certain feature, **TissueFAXS** will display an informative message:

Figure 267 - Example of License Message